SHOUT
SHARE
IT

THE ANNALS OF APPLIED BIOLOGY

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, Manager
LONDON: FETTER LANE, E.C. 4

H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3 AND 4, ARTHUR ST., NEW OXFORD ST., LONDON, W.C. 2
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(Agents for the United States and Canada)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

All rights reserved

THE ANNALS OF APPLIED BIOLOGY

THE OFFICIAL ORGAN OF THE ASSOCIATION
OF ECONOMIC BIOLOGISTS

EDITED BY
W. B. BRIERLEY
AND
D. WARD CUTLER
WITH THE ASSISTANCE OF THE COUNCIL

VOLUME IX

MARINE BIOLOGICAL LABORATORY
WOODS HOLE
LIBRARY
MASS.

CAMBRIDGE
AT THE UNIVERSITY PRESS
1922

M96(1)

PRINTED IN GREAT BRITAIN

CONTENTS

No. 1 (April, 1922)

PAGE

1. The Control of the Greenhouse White Fly (*Asterochiton vaporariorum*) with Notes on its Biology. By Ll. Lloyd, B.Sc. (Leeds). (With 5 Text-figures, 2 Diagrams and Plates I and II) . 1
2. Observations on the Ensheathed Larvae of some Parasitic Nematodes. By T. Goodey, D.Sc. (With 1 Text-figure) . . 33
3. Leaf Character in Reverted Black Currants. By A. H. Lees, M.A. (With 46 Text-figures and 11 Graphs) 49
4. Further Observations on *Sitones lineatus* L. By Dorothy J. Jackson, F.E.S. (With 2 Text-figures) 69
5. Contributions to the Biology of Freshwater Fishes. I. The Effects of various Impurities in a Stream on the Life of Spermatozoa of Trout and Young Trout. II. Biological Problems connected with a Trout Farm. By W. Rushton, A.R.C.S., D.I.C., F.L.S. 72
6. Obituary Notice. Dr Caroline Burling Thompson, 1869–1921 . 81
7. List of Members of the Association of Economic Biologists for 1921–2 83
8. Laws of the Association of Economic Biologists 90

No. 2 (June, 1922)

1. Bionomics of Weevils of the Genus *Sitona* injurious to Leguminous Crops in Britain. Part II. *Sitona hispidula* F., *S. sulcifrons* Thun and *S. crinita* Herbst. By Dorothy J. Jackson, F.E.S. (With 5 Text-figures and Plate III) 93
2. "Sleepy Disease" of the Tomato. By W. F. Bewley, D.Sc. (With Plates IV–VII) 116
3. Biological Studies of *Aphis rumicis* Linn. Reproduction on Varieties of *Vicia faba*. By J. Davidson, D.Sc. With a Statistical Appendix by R. A. Fisher, M.A. (With 1 Text-figure) . 135
4. The Toxic Action of Traces of Coal Gas upon Plants. By J. H. Priestley 146
5. Common Scab of Potatoes. Part I. By W. A. Millard, B.Sc. (With Plates VIII, IX) 156
6. Additional Host Plants of *Oscinella frit*, Linn. among Grasses. By Norman Cunliffe, M.A. (Cantab.) 165
7. Studies in Bacteriosis. VI. *Bacillus carotovorus* as the cause of Soft-Rot in Cultivated Violets. By Margaret S. Lacey . 169
8. Review 171
9. Report of the Council 173
10. Obituary. A. W. Bacot 175

Nos. 3 and 4 (November, 1922)

PAGE

1. A Study of the Life-History of the Onion Fly (*Hylemyia antiqua*, Meigen). By Kenneth M. Smith, A.R.C.S. (With Plates X and XI) 177
2. The Smut of Nachani or Ragi (*Eleusine coracana* Gaertn.). By G. S. Kulkarni. (With 2 Text-figures) 184
3. On the Young Larvae of *Lyctus brunneus* Steph. By A. M. Altson, F.E.S. (With 2 Text-figures) 187
4. Effect of High Root Temperature and Excessive Insolation upon Growth. By Winifred E. Brenchley, D.Sc., assisted by Kharak Singh, M.A. (With 2 Text-figures) . . . 197
5. Studies in Bacteriosis. VII. Comparison of the "Stripe Disease" with the "Grand Rapids Disease" of Tomato. By Sydney G. Paine and Margaret S. Lacey 210
6. The Infestation of Fungus Cultures by Mites. (Its Nature and Control together with some Remarks on the Toxic Properties of Pyridine.) By Sibyl T. Jewson, M.Sc. and F. Tattersfield, B.Sc., F.I.C. (With 4 Text-figures) 213
7. On the Development of a Standardised Agar Medium for counting Soil Bacteria, with especial regard to the Repression of Spreading Colonies. By H. G. Thornton. (With 13 Text-figures) 241
8. Studies on the Apple Canker Fungus. II. Canker Infection of Apple Trees through Scab Wounds. By S. P. Wiltshire, B.A., B.Sc. (With Plate XII) 275
9. The Insect and other Invertebrate Fauna of Arable Land at Rothamsted. By Hubert M. Morris, M.Sc., F.E.S. (With 7 Text-figures) 282
10. On the Life-History of "Wireworms" of the Genus *Agriotes* Esch., with some notes on that of *Athous haemorrhoidalis* F. Part III. By A. W. Rymer Roberts, M.A. (With 1 Text-figure and Plates XIII, XIV) 306
11. The Accuracy of the Plating Method of estimating the Density of Bacterial Populations, with particular reference to the use of Thornton's Agar Medium with Soil Samples. By R. A. Fisher, M.A., H. G. Thornton, B.A., and W. A. Mackenzie, B.Sc. (With 2 Text-figures) 325

VOLUME IX APRIL, 1922 No. 1

THE CONTROL OF THE GREENHOUSE WHITE FLY (*ASTEROCHITON VAPORARIORUM*) WITH NOTES ON ITS BIOLOGY[1].

BY LL. LLOYD, D.SC. (LEEDS),

Lately Entomologist at the Experimental and Research Station, Cheshunt.

(With 5 Text-figures, 2 Diagrams and Plates I and II.)

CONTENTS.

	PAGE
1. Introduction	1
2. Acclimatisation	1
3. Food plants	4
4. Habits of adults	5
(1) Length of life	6
(2) Fecundity	6
(3) Mating	7
(4) Parthenogenesis	8
5. Development	8
(1) Egg	8
(2) Scale	9
6. Occurrence of *A. sonchi* Kotinsky in England	15
7. Economic importance	16
8. Control	17
9. Fumigation	18
(1) Naphthalene	18
(2) Tetrachlorethane	20
(3) Tobacco preparations	21
(4) Cyaniding	21
(5) Poster on Cyaniding Tomato Houses	31
10. Summary	31
References	32
Explanation of Plates	32

1. INTRODUCTION.

THE classification of the Aleyrodidae has been recently revised by Quaintance and Baker(1) who have referred *Aleyrodes vaporariorum* Westw. to the genus *Asterochiton* Maskell. The insect, popularly known as the Greenhouse White Fly, or Snow Fly, is thought to be a native of Brazil, but is now widely distributed. Its fecundity and polyphagous habit have rendered it one of the worst greenhouse pests and it is responsible for the loss of large sums every year in the British Isles.

2. ACCLIMATISATION.

In England it breeds freely in the summer out of doors on a wide variety of plants, shrubs and trees in the neighbourhood of infested greenhouses. The vast swarms found around these in the summer and autumn owe their origin mainly to the adults which are continually passing out of the greenhouses and, to a less extent, to those which have been fostered in cold frames over the winter. At the same time it is highly probable that the insect can survive mild winters out of doors in the south of England and it can certainly do so in the Channel Islands where it is a more serious pest than in the Lea Valley. In the winter of 1919–20, when the insect was being bred for the purposes of this study, the adults could be found around the experimental house throughout, especially on *Althaea rosea* and *Aquilegia*. The first emergence of the adults of the insects breeding outside was observed on July 1. Through the summer and autumn they were excessively numerous in the garden and became a serious pest on runner beans. At the beginning of December the greenhouses and cold frames were free from the pest but the adults were still to be found on *Digitalis* and *Althaea* where a batch of recently laid eggs was seen. Unfortunately the leaf which held these perished. In early December there was a week of snow with 23 degrees of frost on one occasion. Two adults were seen alive after this. The latter half of the month was cold and wet and no more of the insects were found in

---

[1] A grant in aid of publication has been received for this communication.

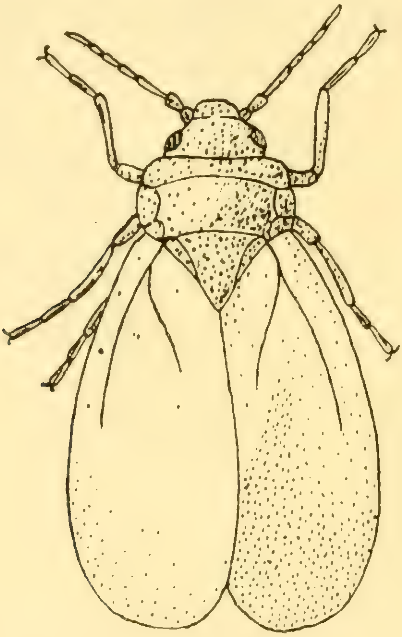


Fig. 1. Adult *A. raporariorum* (× 100).

the garden till the end of May when a few adults were seen on *Althaea* and *Philadelphus*. The Station houses were still quite free from the pest and the nearest infested greenhouse was 200 yards away from the garden. These were probably, though not certainly, survivors from the heavy infestation of the previous year.

Experiments carried out in the winter 1919–20 afford evidence that the insect may survive a mild winter out of doors in small numbers in the Lea Valley.

An *Urtica dioica*, heavily infested with all stages of the pest, was enclosed in a capacious muslin sleeve and placed outside on January 15. The adults became gradually reduced in numbers until on April 13 only 12 survived and the young foliage held numerous eggs. All the scales on the plant were dead.

A second nettle plant, heavily infested with all stages, was cleared of adults, enclosed in muslin, and placed outside on February 2. Seven adults emerged between February 13–20, and eight more between March 9 and April 13. On the latter date oviposition was occurring, but there were no intermediate stages between egg and adult. On May 20 the plant held a few adults, eggs and first stage larvae only.

A *Lamium* was subjected to a massive infestation of adults on January 14; the following day, when it was well covered with eggs, the flies were cleared from it and it was sleeved and placed outside. The eggs commenced to hatch after 87 days on April 10 and continued to do so until May 10, 117 days after oviposition.

Adults were placed in muslin-covered glass vessels containing moist soil with and without cut foliage and placed in the shade outside in January. It was found that where no foliage was enclosed they died in less than a month, January 15 to February 9 (25 days) being the longest period that one survived. In one case a *Lamium* leaf was enclosed and this kept curiously green and fresh for nearly three months. Seventeen adults were placed with it on January 15 and lived an average of 36 days, five survived 50 days, and the last one died on April 6, the 82nd day.

The outside shade temperatures during this period will be found in Table I. Although the winter was a mild one all the insects concerned in these experiments experienced frost and the adult which survived 82 days was subjected to frost on 25 nights. It is therefore clear that both the eggs and the adults are able to withstand considerable cold, but that the intermediate stages are less resistant. Both the resistant stages are dependent on living foliage; as the adult, when subjected to the alternate cold of night and warmth of day, requires food, and the eggs on foliage severed from the plant shrivel and die. Even were this not the case the larva has not that power of movement enabling it to pass on to living plants.

In *Al. citri* the wintering stage is the pupa(2) and the phenomenon of partial brooding, such as is familiar in the case of many Lepidoptera, occurs, some of the pupae going into the wintering condition quite early in the season and the proportion which so delay emergence increasing as the cold weather approaches. No such partial brooding occurs in *Ast. vaporariorum* and its dependence on living foliage shows that it is not fully adapted to a temperate climate where the occasional occurrence of severe winters, when all foliage except that of leathery evergreens is cut down, must exterminate it out of doors.

Table I. *Showing shade temperatures* (*degrees F.*) *in greenhouse and outside during investigation.*

	Outside		Greenhouse	
	Mean	Range	Mean	Range
1919				
December	41·2	24–53	60·6	48–76
1920				
January	39·2	21–54	62·9	50–87
February	41·4	23–54	63·2	45–83
March	46·0	26–73	66·3	43–100
April	50·5	29–71	66·8	44–101
May	55·2	27–86	74·7	51–101
June	59·9	33–87	71·5	47–96
July	60·0	41–84	71·1	53–92
August	58·2	41–83	70·4	49–100
September	57·7	36–80	—	—
October	49·0	23–76	—	—
November	42·2	20–58	—	—
December	39·9	9–53	—	—

3. FOOD PLANTS.

The insect has a wide range of food plants but those which suit it best have rather thick sappy leaves and among its most favoured hosts may be mentioned the following: tomato, potato, cucumber, vegetable marrow, French beans, tobacco, hollyhock, calceolaria, dahlia, heliotrope, stinging nettle. On these plants practically every egg laid produces an adult under favourable circumstances. On a number of hard leaved plants it can breed successfully but the mortality of the larvae is great and the plants do not frequently become massively infested. Such plants are the grape vine, various fuchsias, *Calla*, begonias, geraniums.

On the younger foliage of the tuberous begonias none of the scales survived the first moult and on the older leaves scales at the extreme periphery alone reached maturity. A similar thing occurred on fuchsias of the Mrs Marshall type where frequently a leaf was found with a complete fringe of mature or empty scales while on the rest of the leaf all the scales were dead. On chrysanthemums breeding was free on old foliage but not common on young growth. On two weeds strongly favoured by the adults, *Solanum dulcamara* and *Lamium purpureum*, no scale was ever found to mature, all dying either before or just after the first moult. On narcissus, tulip, hyacinth and various grasses eggs were often laid, but no larvae passed the first moult. Mature scales have been found rarely on elder and hawthorn and rather frequently on elm. This list by no means exhausts the food plants of the insect which were noted, but is merely indicative of its range.

4. HABITS OF ADULTS.

The adults usually mate on the leaf on which they emerge and frequently commence oviposition there. Later they seek younger foliage. Outside, when there is a perceptible wind, they are very reluctant to take flight, but on warm still days they may sometimes be seen hovering in numbers over their host plant. In the tomato houses they often remain very localised until the infestation on a few plants has become massive. Trimming the plants and the consequent disturbance aids their dispersal. They are distinctly gregarious as the following figures show, the counts being made in each case on foliage on which no adults had emerged.

On July 8, large bushy *S. dulcamara* growing under staging in the greenhouse, 260 leaflets all young and tender, plant held 90 flies distributed on 35 leaflets and of these 15 (16·6 per cent.) were on one leaflet and 9 (10 per cent.) on another.

On July 16, 10 plants, *Trifolium sativum*, growing in a box in the greenhouse held 242 flies distributed as follows: 7, 4, 35. 79 (33 per cent.), 16, 11, 3, 0, 53, 34.

On July 20 the top 40 leaves of an *Al. rosea* held 129 flies of which 55 (43 per cent.) were on the 22nd leaf from the top.

On August 27, a young *Urtica* held 32 flies of which 22 (68 per cent.) were on one leaf and the others distributed over the remaining 11 leaves.

This gregarious habit has possibly originated for the better protection of the scales from parasites. If a healthy scale is watched under a moderately high power of the microscope and in bright sunlight, the "honey dew" excreted at the anus in the base of the lingula is seen to form into bubbles which swell up very suddenly and burst, distributing the dew as a fine spray. The mechanism of the bubble formation has not been made out. There is at the caudal end of the vasiform orifice a small trumpet-shaped organ and the tip of the lingula frequently touches the mouth of this. The trumpet-shaped organ lies at the end of the furrow running from the caudal air channel of the scale to the vasiform orifice. No actual air channel was followed, and time did not permit of any exhaustive examination of structure. A large number of scales blowing bubbles in this way would form a continuous shower of a sticky spray and it seems reasonable to suppose that this would be a deterrent to the small parasitic hymenoptera. Bubble formation is well known in another group of the Hemiptera, certain Cercopidae, the well-known "froghoppers" which form the "cuckoo spit."

The adults exhibit a remarkable colour reaction, being strongly attracted to yellow, and to green and orange in proportion to the amount of yellow these colours contain. The experimental work on this subject will be recounted elsewhere.

(1) *Length of life.* A study of the length of life of the adults, their fecundity and parthenogenesis was carried out with newly emerged females, alone or with single males, on small plants growing in muslin covered beakers. In this way daily observations could be made without disturbing the insects and these could be transferred to fresh uninfested plants before their offspring attained maturity. Unfortunately *Lamium purpureum*, a plant which thrives well under these artificial conditions, was used in a number of the earlier experiments and as none of the young matured these were largely wasted.

The average life of 16 females, including three which came by accidental deaths, was 40 days. The longest life recorded was 104 days, on *Lamium*. The average life of 10 males was 25 days, the longest being 46 days, also on *Lamium*.

(2) *Fecundity.* The average number of eggs laid was 130 per female and the rate of oviposition averaged about three eggs a day. The largest number laid was 534 giving an average of slightly more than five a day. There appeared to be some variation in fecundity in accordance with the food plant, such as Morrill and Back (2) describe for *Al. citri*, but the experiments were insufficient for this to be certain. Oviposition began on the second to fifth day after emergence in most cases. The high mortality of the young, which occurred several times on suitable foods, was due to the difficulty of keeping all the foliage healthy under conditions ensuring that no invading insects could contaminate the experiments.

Table II. *Showing the length of life, fecundity, and sex of offspring of* A. vaporariorum, *mated and unmated.*

Experiment No.	Date commenced	Plant	Condition of female	Life in days		Number of eggs	Offspring	
				Male	Female		Males	Females
5	20. I.	*Ranunculus*	Virgin	—	9 + (escaped)	4	3	0
6	26. I.	*Lamium purpureum*[1]	,,	—	71	186 ⎫ 271	—	—
		Urtica dioica[2]				85 ⎭	76	0
8	26. I.	*Lamium purpureum*[1]	,,	—	77 + (escaped)	147 ⎫ 297	—	—
		Urtica dioica[2]				150 ⎭	107	0
9	26. I.	*Lamium purpureum*[1]	,,	—	17	82	-	—
21	6. II.	Tomato	,,	—	46	110	73	—
40	12. IV.	*Urtica dioica*	,,	—	22	88	8	—
7	26. I.	*Urtica dioica*	Mated	8	55	191	33	123
10	27. I.	*Lamium purpureum*[1]	,,	46	104	534	—	—
11	27. I.	*Senecio vulgaris*	,,	7	20	12	0	1
20	6. II.	Tomato	,,	34	39	74	16	12
22	6. II.	*Trifolium pratense*	,,	45	47	83	14	13
25	18. II.	*Urtica dioica*	,,	33	33	102	11	11
26	18. II.	,,	,,	13	14	28	15	8
33	12. III.	,, (out of doors)	,,	40	95 + (drowned)	95	No record	
39	12. IV.	*Solanum dulcamara*[1]	,,	5	13	49	—	—
41	20. IV.	*Urtica dioica*	,,	16 + (killed)	16	63	2	8

[1] Food plant unsuited to larvae.

[2] Transferred to this plant.

(3) *Mating.* Mating occurred soon after emergence and it is the habit of the male to rest quietly by the side of the female and to effect impregnation repeatedly. In one case coitus was observed between the same pair on five occasions between January 27 and February 26 and probably also on March 6. Notes were made of their relative positions

on 37 days and on 23 of these they were close together. This repetition appears to be unnecessary as in one case when coitus occurred on January 29 and the male died on February 3 the still isolated female continued to oviposit until March 19 and the effect of the fertilisation was evident to the end in the sex of her offspring, the eggs laid from January 30 to March 2 producing 82 females and 21 males and those laid from March 2–19 producing 41 females and 12 males.

(4) *Parthenogenesis.* Morrill first noted parthenogenesis in Aleyrodidae and in conjunction with Back(2) found that unfertilised eggs of *Ast. vaporariorum*, *Al. citri* and *Al. nubifera* always gave rise to male offspring. Hargreaves(3), working in England, found just the reverse, and bred two generations of females of *Ast. vaporariorum* in the absence of males and states "out of the hundreds of flies that I examined I did not encounter a single male." Williams(4) found several colonies of the insect in England consisting entirely of females or in which this sex largely predominated. In his experiments in breeding he obtained from mated females small families in which the sexes were equal but none of the offspring of his virgin females reached maturity. This author and later Schrader(5) discuss the genetics of the insect and the suggestion is made that the parthenogenetic female producers in England may have arisen by mutation from the American parthenogenetic male producers of America and that the occurrence of some males in England may be due to fresh importations.

In the greenhouses in the Lea Valley the sexes occur in approximately equal proportions, counts giving in July out of 305 insects a male percentage of 52·8 and in October out of 118 a male percentage of 46·6. The breeding experiments show that the strain agrees with the American race in this important point, as the data in Table II show. Five virgin females produced offspring totalling 267 all of which were males. Seven mated females produced offspring also totalling 267 and of these 91 (34 per cent.) were male and 176 female.

5. DEVELOPMENT.

(1) *Egg.* The eggs (Fig. 2) (Plate I, fig. 1) are laid in circles on smooth leaves, but on hairy leaves like those of the tomato they are scattered in groups. A firm attachment to the leaf is gained by means of a short stalk which rests in a cut made by the female. Like all the other stages of the insect they are covered with wax. At first they are greenish yellow in colour but darken in two or three days in warm weather and during the greater part of the incubation period they are quite black.

They are almost invariably placed on the undersides of the leaves. As indicated above the incubation period may be very prolonged in cold weather outside. The varying incubation periods were recorded on a series of plants from December to August and the results are summarised in Table III. The longest period observed outside was 117 days and the shortest 13–16 days in August, mean temperature 58° F. The incubation period outside in August is little more than half that recorded in the greenhouse in December, though the mean temperature in the latter case was two degrees higher and is approximately the same as that in April under glass with a mean temperature of 67°. This seems to show that sun heat is more stimulating than artificial, the sunshine hours in the three months being: December, 27; April, 87; August, 158.

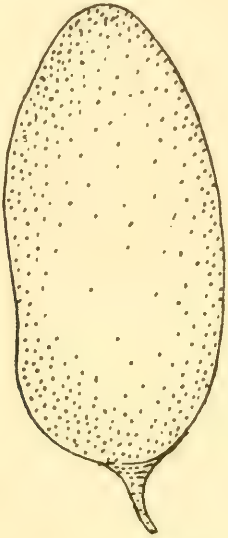


Fig. 2. Egg of *A. vaporariorum* (×350).

(2) *Scale.* The characteristics of the four scale stages are described by Hargreaves(3). The first larva (Fig. 3*a*, *b*) moves about on the surface of the leaf but usually only a sufficient distance for it to grow without coming in contact with others from the same batch of eggs. The movement was usually confined to a few hours and once only was one seen to move the day after hatching. On one occasion a larva was seen walking on the stem of a plant, a very small *Trifolium pratense*, the leaves of which were overcrowded with scales. When cut foliage heavily infested with hatching eggs was placed on the soil around the stems of *Urtica*

and beans no migration of the larvae from the dead leaves to the living plants occurred. When eggs were hatching in numbers on the unsuitable foot plant *Lamium*, and it was particularly desired to preserve the larvae, an *Urtica* was planted by the side of the former and the leaves of the two were stitched together. No larvae passed from the unsuitable to

Table III. *Showing the duration of the egg and scale stages of* A. vaporariorum *on various plants in a heated greenhouse and outside.*

	Plant	Date of oviposition	Date of hatching	Duration of egg stage (in days)	Date of emergence of adults	Duration of scale stage (in days)	Number emerged
In heated greenhouse	Tomato	3–4. xii.	23–29. xii.	20–25	20–28. i.	28–30	Many
	Urtica	3–4. xii.	23–29. xii.	20–25	21–28. i.	29–30	,,
	Anemone (St Brigid)	14–16. i.	1–4. ii.	17–21	9–18. iii.	37–43	28
	Bean	28–30. i.	14–19. ii.	17–20	18–31. iii.	33–41	260
	Tulip	20–23. ii.	11–15. iii.	20–21	—	—	0[1]
	Bean	23–24. ii.	13–15. iii.	19–20	7–17. iv.	25–33	49
	Begonia (tuberous)	2–3. iv.	16–19. iv.	14–16	—	—	0[2]
	Urtica	2–3. iv.	16–18. iv.	14–15	12–18. v.	26–30	87
	Potato	23–25. iv.	5–8. v.	12–13	26. v.–4. vi.	21–27	270
	Tomato	12–13. v.	21–22. v.	9	8–18. vi.	18–27	870
	Bean	9–10. vii.	17–19. vii.	8–9	3. viii.?	17–?	Many
Outside	*Lamium*	12–15. i.	10. iv.–10. v.	87–117	—	—	0[1]
	Urtica	5–8. iii.	28. iv.–14. v.	54–67	—	—	0[3]
	Urtica	2–3. iv.	14. v.–20. v.	42–48	—	—	0[3]
	Urtica	5–19. iv.	20. v.–1. vi.	43–45	29. vi.	40	5
	Bean	—	8–10. viii.	—	7. ix.–?	30–	Many
	Bean	2–3. viii.	15–18. viii.	13–15	15–20. ix.	30–33	Many

[1] Food unsuited to larvae.

[2] Young begonia foliage unsuited to larvae.

[3] All infested leaves shed.

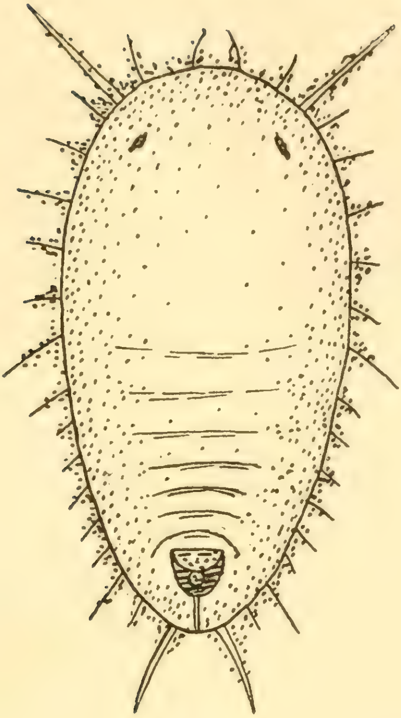


Fig. 3 *a*.

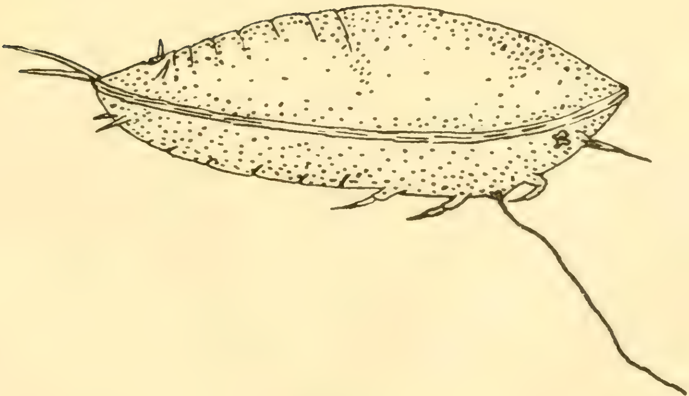


Fig. 3 *b*.

Fig. 3. First stage larva of *A. vaporariorum*, recently hatched (× 300). *a*. dorsal view, *b*. lateral view.

the suitable food. The movement of the larvae is therefore not a migratory one and when the eggs are laid on an unsuitable plant or the foliage holding them is severed from the plant, they cannot survive. Eggs and feeding larvae on severed foliage shrivel up and die with the drying of the foliage which holds them.

All the larval stages are distinctly flat after the moult and the growth in each stage is in depth only. The first three become very turgid towards the moult and the skin splits at the junction of the thorax and abdomen by a T-shaped orifice. Through this the larva protrudes the head and thorax and forces itself forward till it can grasp with its legs the leaf in front of the old skin. It then elevates the posterior end of the body thus tearing the old skin away from the leaf. This is heavy through being filled with the "honey dew" and when it is released it falls clear of the leaf as a rule. Walking subsequent to a moult has not been observed but when the scales are crowded a revolving motion is often seen while the larva feels for a clear space. Overlapping of the scales, however, is common in heavy infestations. When the adult emerges from the mature scale the empty shell is left attached to the leaf.

The fourth stage of the scale (Fig. 4) is always referred to in the literature of the Aleyrodidae as the pupa, but at the beginning of the instar it is as much a larva as the preceding stages. Its dorsal skin becomes somewhat heavily chitinised and leathery and in its growth this is elevated entire from the leaf, a corrugated palisade of wax forming as the elevation proceeds. The stout waxen case is continued entirely over the ventral surface and the mouth stylets protrude through it. Respiration takes place at folds where the otherwise translucent wax remains opaque white and porous. These breathing folds are the same depth as the palisade and are situated one median posterior and two antero-lateral in the region of the thorax. The dorsal surface carries a marginal fringe of short tooth-like waxen processes arising from bosses. These short spines curl downwards. There is also a system of longer waxen processes standing upright from the scale. Hargreaves mentions and figures eleven pairs of which seven pairs are marginal. In the specimens examined during this work only four pairs could be distinguished from the marginal teeth, viz. one anterior, one over the lateral breathing folds, one posterior to this pair at the level of the first abdominal segment, and one caudal, while in the typical form those on the disc agreed with Hargreaves' description, viz. one pair cephalic, one thoracic, one on the third and one on the fourth abdominal segments. In a few of the specimens mounted from tomato the fifth and sixth abdominal segments also bear spine bosses, and in one case there is an asymmetrical one on the fifth segment. Where these additional ones are not developed, minute hairs can be seen replacing them, evidently vestiges.

This instar becomes quite opaque early in its development and it is therefore not possible to tell by the movements of the pharyngeal pump how long it continues to feed. After the eyes and other organs are well developed, if it is removed from the leaf its mouth stylets wave to and fro as though they were still functional. Considerable growth in depth also takes place after the adult eyes are distinct. If it is removed from the leaf shortly before emergence the mouth stylets break off short by the case and have evidently ceased to function except as an anchor. About this time a copious excretion of honey dew takes place and the insect then lies tolerably free inside the old larval skin.

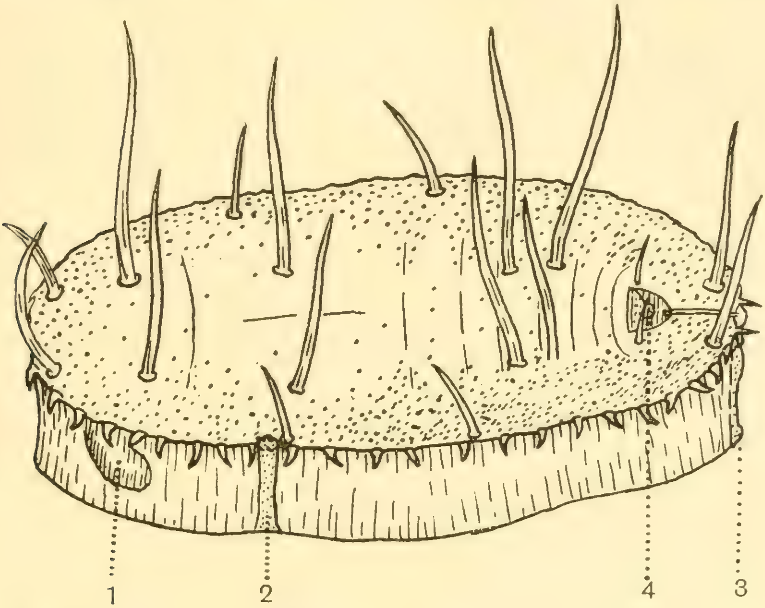


Fig. 4. Mature scale (pupa) of *A. vaporariorum* (× 130). 1. adult eye, 2. thoracic breathing fold, 3. caudal breathing fold, 4. vasiform orifice.

The duration of the scale stage was noted on a variety of plants which were examined before use to ascertain that they were free from the pest. They were then heavily infested with the fly for one or two days. After this the fly was cleared from them and they were enclosed in muslin sleeves. The hatching of the eggs and the emergence of the adults were noted. The limits of the scale stage in the greenhouse were found to vary from 45 days in February to 17 days in July and bore a close correspondence to the temperature (see Tables I and III). There was however some variation with the different species of plants, since in one experiment when a variety of plants were infested on April 23–25 and the eggs on all hatched from May 5–8 the duration of the scale stage was on fuchsia 26–31 days, on thick-leaved zonal geranium 24–30 days and on tomato, potato, calceolaria, and thin-leaved variegated geranium 21–29 days.

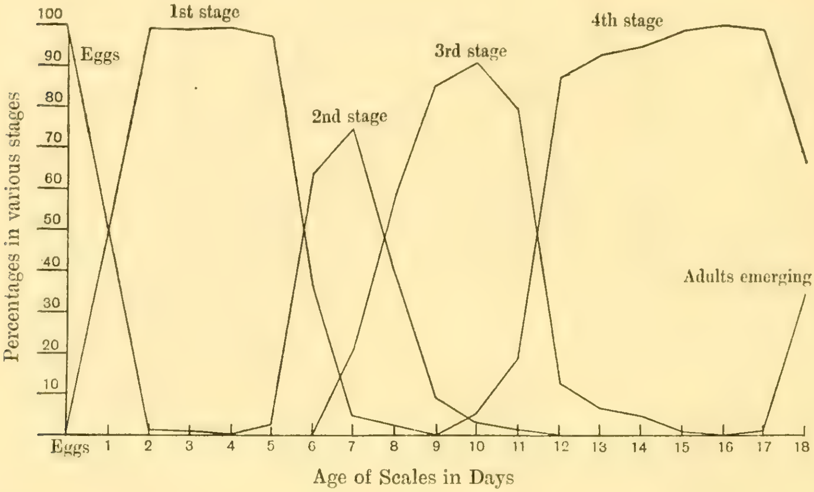


Diagram I. Showing the time spent in the four larval stages by *A. vaporariorum* on French beans at optimum temperature (mean 74° F.). The maximum number moult where the curves cross at 50 %.

In July young bean plants, known to be free of the insect. were taken one each day for 19 days and exposed for 24 hours to a large number of adults. These were then cleared off and the plants were kept in a fly-free muslin cage. When emergence of adults commenced on the plant first infested the series was stopped, each plant holding developmental stages of the same age from egg to adult. A portion of each plant holding 200–450 scales was then examined and each scale was assigned to its particular instar, the condition of 4900 scales being thus noted. The percentages in the various stages on each plant are shown in Diagram I. The mean temperature during the experiment was 74° F. with a range of 54–193°. Hatching began on the 8th day after oviposition and was practically complete by the 10th day. The figures showed that on the average the duration of the first stage was 5 days, the second 2 days, the third 3 days, and the fourth stage 8 days. On *Ranunculus* in February and March with a mean temperature of 64° (range 45–94°) the duration in days of the four stages in four scales was: 1st, 6–7; 2nd 4–6; 3rd 8–11; 4th 16–19. On beans in March and April, with a mean temperature of 67° (range 47–101°), the average duration in days of 49 scales was: 1st, 7; 2nd, 3; 3rd, 6; 4th, 12. These instances sufficiently show the proportion of the larval life which is spent in the various instars.

The emergence of the adults appeared to be always in the early hours of daylight.

6. OCCURRENCE OF *A. SONCHI* KOTINSKY IN ENGLAND.

A second form of pupa appeared in the greenhouse where the main culture of *A. vaporariorum* was kept (Plate I, fig. 2). This scale differed from the typical form in the absence of dorsal tubercles and processes and the greater development of the marginal waxen processes which stuck out parallel with the leaf surface. It was first seen on a small *Acer* which had been brought in to test its susceptibility to the fly and had been in the greenhouse for some weeks; later, on *Brassica oleracea* which had been introduced for the same purpose. Both these plants were thought to be clean at their introduction and the typical scales never developed on them. It was also found on *Polygonum aviculare* and *Sonchus oleraceus* which had grown from seed in the chamber. Outside it was found on *Acer*, *Sonchus*, *Clarkia*, *Tropaeolum indicum* and *T. canariense*. Its incidence in numbers in the Station garden corresponded to that of the typical *A. vaporariorum*, the experimental greenhouse being the focus. The same scale was seen in Guernsey in October massively infesting *Sonchus* in the greenhouses, again in association with *A. vaporariorum*. A *Polygonum* which, after pocket-lens examination, was believed to hold the atypical form only was cleared of all adults and placed in a muslin cage with three tomato plants which were uninfested. This cage was unopened for a month during my absence from the Station, watering being done through the muslin. At the beginning of October when the cage was opened there had been time for one generation to breed through. The tomato plants were found to be massively infested with typical *A. vaporariorum* in all stages. It was then thought possible that the two scales belonged to the same species as no differences in the adults could be detected. Material from the cage and from outside was therefore submitted to Mr Laing of the British Museum who reported:

"The absence of dorsal tubercles gives the form found on *Acer*, *Tro-*

paeolum and also to a certain extent on *Polygonum aviculare*. This form may be that originally described by Barensprung as *complanatum*, but his description is totally inadequate. It almost certainly is *sonchi* of Kotinsky from Hawaii, and the absence of the two tiny caudal spines would make it *tentaculatus* Bemis, from California. On *P. aviculare* I find in my preparations both typical and atypical pupa cases, but on the tomato the typical form seems to be present alone. Is it not possible that the two forms were present at the beginning and that only the typical form developed on the tomato? The case would then be very similar to Morrill's experiment when he separated *packardi* Morrill from *vaporariorum*. In addition to the absence of the dorsal tubercles in the atypical material there are other minor differences and seeing the material without knowing anything of the experiment I should unhesitatingly have called the two forms distinct species."

It was intended to do further work with the atypical form in the present summer but circumstances have prevented this. Its wide distribution, Hawaii, Guernsey, the Lea Valley, and possibly California, and its apparent association in two of these with *A. vaporariorum* make it a very interesting form.

7. ECONOMIC IMPORTANCE.

Financial loss caused by *A. vaporariorum* results mainly through its attacks on tomatoes, beans and potatoes grown under glass. The two latter suffer less than the former because they are brief crops and the insect does not have the same opportunity of causing massive infestations on them for this reason. The damage is partly direct through loss of sap, but is mainly due to the layer of honey dew which soon covers the foliage and fruit of the infested plants. In this medium sooty moulds grow, forming a black felt over the foliage which keeps away sunlight, while all fruit from infested plants must be wiped before it can be marketed. The fungus growth on the tomato plants was examined by Dr W. F. Bewley, who found it to consist of *Penicillium* sp. predominating, together with *Cladosporium herbarum* and *Fumago vagans*, all saprophytic forms.

In order to estimate the damage done by an unchecked attack on tomatoes, 34 young plants in 12-inch pots were lightly infested with about ten adults each on May 6 and placed in a chamber in the greenhouse. On half of them the pest was allowed to develop unchecked while the others were removed about once a fortnight to another chamber and fumigated with hydrocyanic acid gas in order to keep the infestation under control. Apart from the fumigations the plants received the same treatment. The infested plants were practically dead on August 24 and the last fruit was picked from them. Their total yield was 29 lbs. 14 oz. The fumigated plants were still vigorous at the last fruit picking on October 8 and their total yield was 66 lbs. 8 oz. or about 4 lbs. of fruit per plant. Not uncommonly tomato plants in trade nurseries are as badly attacked as these and it may be stated fairly that if the plants are infested early and the infestation is not checked a loss of more than half the crop will be the result.

Such a condition only obtains when the grower deals in mixed crops keeping his houses occupied during the winter. The grower who only grows tomatoes has his houses empty for several months and nearly always commences the season free from the pest. Invasion by the insects is liable to occur in May and June and it is not until the late summer that fumigation becomes necessary.

Although the cucumber was mentioned above among the favourite foods of the insect, trade growers of this crop do not recognise it as a pest in the Lea Valley. It is sometimes present in the houses early and late in the year, but generally disappears when the weather becomes warm. In the tomato houses it was observed that when the temperature of the air approaches 100° F. the flies become restless and flutter up to the glass, many escaping through the laps. It was found by experiments in thermostats that they are stupified when the temperature rises to 105°. Fifty flies so stupified by 40 minutes' exposure to 102–106° nearly all recovered by the second day after the experiment. One out of 20 recovered after 5 minutes' exposure to 104–110°. It was clearly impracticable to effect control of the pest in the tomato houses by so raising the temperatures so no further details of the series of experiments will be given, but they showed that it is the atmospheric conditions of the cucumber houses which often rise above 100° that prevent the white fly from becoming a serious pest there.

8. CONTROL.

The spread of the insect is greatly aided by the culpable negligence of some nurserymen who make a business of the sale of young plants. Bedding plants such as geraniums and salvias are often sold infested with the pest, and even young tomato plants are sometimes sent out in a similar unclean state. Probably only legislation could stop this dangerous practice, but growers may be advised to ask for a guarantee of cleanliness in this respect for any plants they purchase for gardens around the nurseries, and especially for young tomato plants. It is always advisable to raise tomato plants from seed rather than to buy them from mixed growers. Propagation of the new season's crop from cuttings taken from old plants has also led to very serious infestations and though this is not a common practice it is well to warn against it.

Growers should realise that they are themselves responsible for the heavy infestations outside the nurseries, and they should prevent these by never allowing conditions inside to get bad. Very many of the insects pass out of the houses of their own accord but still larger numbers are taken out on trimmings and on the plants at the end of the season. The pest should be kept under such control that the trimmings are never heavily infested and if the plants are still infested when they are cut out at the end of the season, they should be cyanided before removal. It is a common practice to burn sulphur in the houses before the plants are removed, but this is not a good fumigant against the white fly.

The grower of mixed crops should free his nursery from the pest during the winter by fumigations of all the occupied greenhouses and cold frames. A greenhouse may be easily cleaned without fumigation by completely emptying it and digging it over to bury any infested weeds or leaf fragments holding pupae, leaving it empty with the heat on for a week to starve any adults that remain and then reoccupying it with clean plants. Much of the trouble with this pest, especially in Guernsey, is caused by propagating tomatoes in houses containing infested potatoes or beans. A small separate propagating house would prevent this early infestation of the seedlings. Attacks of white fly are often caused by sheltering some ornamental plants such as fuchsias in the greenhouses. The specialist in tomato growing should not permit this practice.

9. FUMIGATION.

Spraying is of little use against white fly, while properly applied fumigants give excellent results. Special attention was given to four fumigants, viz. naphthalene, tetrachlorethane, tobacco preparations and hydrocyanic acid gas, and these will be discussed in turn.

(1) *Naphthalene.* This substance forms the basis of a number of proprietary articles sold as exterminators of white fly and attention was given to it for this reason. Naphthalene is sold in various forms as "pure flake" which is a sublimed form; "crude naphthalene," a dark material containing carbon as an impurity and from which most of the tarry acids have been removed; "drained salts" or "whizzed naphthalene," a damp oily product containing a very variable amount of the tarry acids; "undrained salts" or "unwhizzed naphthalene" which

contains relatively larger quantities of phenols. The effect of these varies greatly, but in the proportions in which it is safe to use them they are toxic only to the adults and this makes them unsatisfactory and excessively costly owing to the necessity for repeated applications.

From 50 to 200 adult white flies on foliage were placed in half-gallon glass-stoppered jars and small quantities of pure naphthalene, cresylic acid and phenol, alone and in various combinations, mixed with ash were introduced on watch glasses. Notes were made of the rate at which the insects were stupified and after an hour the fumigants were removed and the jars were left with muslin covers. A count of the mortality was made the day after the fumigation. The results of a small series of fumigations are given in Table IV. It will be seen that the mortality was slight where naphthalene alone was used, heavy with cresylic acid or phenol, but almost total in most cases (10 out of 12) when naphthalene was used in combination with the tarry acids. The rate of stupifaction was in the same proportion as the final mortality, being slow when naphthalene alone was used. A similar series was carried out with various grades of naphthalene, 0·25 grm. in 0·75 grm. of ash being used and the fumigation lasting 70 minutes at a temperature of 66° F. The mortality was as follows:

1.	Naphthalene, pure flake	killed	4/65 = 6·1 %
2.	,, high grade white (Crow & Co.)	,,	1/57 = 1·7
3.	,, crude (carbon impurities)	,,	1/35 = 2·9
4.	,, ,, (another sample)	,,	2/60 = 3·3
5.	,, drained salts (Crow & Co.)	,,	50/62 = 80·7
6.	1 grm. containing 75 % destructor refuse and 25 % volatilisable naphthalene containing some tarry acids (proprietary remedy)	,,	15/89 = 16·8

Table IV. *Showing the percentage mortality of adult* A. vaporariorum *obtained* in vitro *with pure naphthalene alone and with tarry acids, fumigations lasting one hour. Temperature* 69–72° F.

	Naphthalene grms.					
	0	0·1	0·2	0·3	0·4	0·5
No tarry acids	—	—	—	—	—	3·5
	—	—	—	—	—	3·0
Cresylic acid ("pale straw") 0·1 grm.	79	—	—	—	62·6	95
	64	—	—	—	—	—
Pure phenol 0·1 grm.	66	100	99	99	98	97
	39	—	—	—	100	98
	76	—	—	—	—	—
Cresylic acid 0·1 grm. + phenol 0·1 grm.	78	—	—	85	—	100
	75	—	—	—	—	100

It is clear that the effect of naphthalene on white fly depends on the presence of the residue of tarry acids. The finding was checked by fumigations of an infested greenhouse with the various grades, but as it is at best a poor remedy these will not be detailed. The materials give an unpleasant flavour to the fruit and it must be realised that the introduction of an unknown amount of carbolic acid among growing plants is a risky proceeding. The vendors of naphthalenes state that the crude forms contain a very variable amount of tarry acids in the different samples, but they are unable to guarantee the strength. This would make it impossible to standardise a treatment.

(2) *Tetrachlorethane.* This liquid has been occasionally used for greenhouse fumigation during the last two years at the Experimental Station, and gives good results against white fly. Its use is simple as it is merely poured down the centre of the house in the evening and this is kept closed for as long as possible on the following day. As it is not a very poisonous substance it may be used in conservatories opening into dwellings where the employment of cyanide is undesirable and it has a future as a fumigant in very small greenhouses where the measurement of the small quantity of cyanide required is a difficulty. As it costs about ten times as much as cyanide fumigation the trade grower should take little interest in it.

Its action on adult *A. vaporariorum* is doubtless toxic, but it appears to kill the scales through the effect of the vapour on the wax as considerable numbers of the flies attempt to emerge subsequent to the fumigation and die when partially free from the pupa cases. It has no effect on the eggs. It has been used for a wide variety of plants including tomatoes and no damage has resulted except in one case when the foliage of three young sycamores (*Acer pseudoplanatus*) growing in pots turned brown the day after the fumigation and was subsequently shed. It may be therefore that some greenhouse plants would suffer from it. Daylight during the fumigation did not tend to damage and no preparation of the plants appeared to be necessary.

The liquid should be used at the rate of about half a pint to 1000 c.ft. of space and the fumigation should be repeated as with cyanide. The complete success of the fumigation depends on its duration, and the limiting factor to this is the weather as the house can be kept closed longer in dull than in sunny weather. A dull period should therefore be chosen when possible. The mortalities obtained in four experiments, with varying amounts of tetrachlorethane and varying durations, when in-infested plants were sleeved after the fumigations and kept under observation for two to three weeks, are shown in Table V.

Table V. *Showing the mortality of* A. vaporariorum *obtained with tetrachlorethane.*

Capacity of greenhouse	Amount per 1000 c.ft.	Duration	Temperature	Mortality	
				Adults	Scales
2500 c.ft.	1 pint	15 hours	61–85° F.	100%	$(\frac{343}{350})$ 98%
4500 ,,	$\frac{2}{3}$,,	12 ,,	65–70	100	$(\frac{583}{699})$ 83
2500 ,,	$\frac{1}{2}$,,	18 ,,	62–65	$(\frac{28}{29})$ 97	$(\frac{65}{67})$ 97
2500 ,,	$\frac{1}{2}$,,	40 ,,	51–68	100	100

(3) *Tobacco preparations.* It was recently stated by a correspondent in a trade journal that some twenty years ago when white fly first started to give trouble to nurserymen in this country it was only necessary to burn a little tobacco in the greenhouse in order to control it, but that now such preparations only stupify it. Whatever they were once, they are at any rate at present of very little use against the pest as they drive enormous numbers of the insects outside and many of those which fall are merely stupified. They have no appreciable effect on the young stages. The various tests made will not be discussed in detail. They included the burning of "shreds" at five times the strength recommended by the maker, fumigations with a proprietary nicotine and camphor fumigant, also with pure nicotine at the rate of $\frac{1}{8}$ oz. per 1000 c.ft. and $\frac{3}{8}$ oz. with $\frac{3}{8}$ oz. camphor per 1000 c.ft. In each case less than a 50 per cent. mortality of the adults was obtained. These preparations have also become almost prohibitory in cost to the commercial grower.

(4) *Cyaniding.* Fumigation with hydrocyanic acid gas or, as it is generally called, "cyaniding" is the most effective method of controlling the pest and is the only one known sufficiently economical for use in trade nurseries. High grade sodium cyanide, 98 per cent. purity (often described as 130 per cent. in reference to the strength of pure potassium cyanide), is employed, and the gas is generated by means of sulphuric acid. Potassium cyanide and phosphoric acid are sometimes used as alternatives, but they have no advantages and are more costly. The proportions in which to employ the materials are: 1 oz. of sodium cyanide in $1\frac{1}{2}$ fluid ozs. of sulphuric acid diluted with 3 fluid ozs. of water. An error which had become almost universal among Lea Valley growers and in other parts has led to most of the failures in the use of these materials in the past. Fear of the poisonous nature of the gas has led the users to drop the cyanide into the acid enclosed in a sealed envelope or some other type of paper packet. When the acid enters the packet and evolution of the gas commences the pressure prevents the free access of the acid and the heat of the reaction chars the remaining cyanide.

Later, when the packet breaks down, the acid will no longer act on the charred mass. The cyanide should be dropped free into the acid so that the reaction may be brisk and unimpeded. A so-called "Safety Cyanide Package" is on the market and this consists of a metal container the side of which is made of thin zinc foil. An additional amount of sulphuric acid is used and the zinc dissolves away completely so that the acid has free access to the cyanide. There is no scientific objection to this container. There is, however, ample time for the operator to drop loose cyanide into the acid and to move on to the next jar at a slow walking pace without detecting the slightest odour from the gas.

A series of eighty fumigations with this gas was carried out in an experimental greenhouse 18 ft. long by 20 ft. wide, height 11½ ft. to the ridge and 4 ft. to the gutter, capacity 2500 c.ft. The recommendations finally made were checked in blocks of tomato houses in trade nurseries and in greenhouses of mixed plants. Temperature, humidity, duration, time of day, size of dose, and the condition of the plants were all varied. Tomato plants in pots were always included, and some of these were of large growth growing in 12-inch pots, to study the effect of the gas on normal plants. Others, tomatoes or beans, were heavily infested with white fly in all stages and were enclosed in muslin sleeves at the end of the fumigation and were examined every day or two for two or three weeks in order to estimate the effect of the gas on the insect. In general three infested plants were included and placed in different positions and at different heights, but it was found that position made no appreciable difference. A fumigation chamber with a capacity of 350 c.ft. was also constructed, but it was found that results obtained in a chamber are useless when applied to a greenhouse owing to the difference in leakage and its use was abandoned except for a few special experiments.

Temperature. The results confirmed the work of other investigators that a somewhat low temperature (below 60° F.) renders the plants less liable to damage, but in practice as the fumigation of tomato houses is most often done in summer and early autumn the operation has generally to be carried out at a somewhat higher temperature than this. On 30 evenings from May to July fumigations were commenced at dusk and on only two occasions was the temperature of the house below 60° though artificial heat was cut off in nearly every case, and on 11 occasions it was above 65°. As will be shown presently it is possible to counteract the harmful tendency of high temperature by withholding water from the plants. It is exceedingly difficult to make any definite recommendation about temperature as, in the case of tomato plants, very severe damage with the same amount of cyanide has occurred at a temperature of 57° with unprepared plants and none at all at 69° with prepared plants of a similar soft growth. The best advice that can be given to the grower is that he should have his houses cool for the fumigation and carefully prepare the plants beforehand as described below.

The toxicity of the gas for the insect was not found to vary within the mean temperature limits of 47° and 66°, total mortalities being obtained with both at the correct dosage.

Time of day and duration. Every writer on the fumigation of greenhouses with this gas mentions the importance of not commencing the operation till dusk, but growers in many cases persist in starting several hours before sunset. Damage which means practically the death of the plant results. All tissue on which the sunlight falls is seared as though with flame, the growing points are killed and the buds and flowers cut off. The materials are always blamed for this and the operation discredited. Moreover the gas is less toxic in sunlight as the following cases show. A fumigation with $\frac{1}{8}$ oz. cyanide per 1000 c.ft. commenced four hours before sunset and lasted $13\frac{1}{2}$ hours gave a mortality of less than 50 per cent. for adults and negligible for the scales, as contrasted with a usual mortality of 90 per cent. for adults and 75 per cent. for scales in fumigations with this quantity commenced at dusk and lasting 8–11 hours. A fumigation with $\frac{1}{4}$ oz. cyanide per 1000 c.ft. commenced three hours before dusk and lasted 12 hours gave an almost total mortality for adults and 70 per cent. for scales, as contrasted with a usual total mortality for adults and total, or almost total, mortality for scales with the same quantity commenced at dusk and lasting 8–11 hours.

It has generally been the custom to recommend relatively large doses of cyanide with short exposures rather than small doses with long exposures. Sasscer and Borden (6) using $\frac{1}{2}$–$\frac{3}{4}$ oz. cyanide per 1000 c.ft., exposure one hour, obtained with this pest a mortality which was total except for eggs and late pupae. In the course of this work $\frac{1}{2}$ oz. cyanide, 1000 c.ft., exposure one hour, gave 95 per cent. mortality for adults, had practically no effect on late pupae, and destroyed about half the younger scales. The same amount, $1\frac{1}{2}$ hours' exposure, gave 100 per cent. mortality for adults and about 90 per cent. for scales. $\frac{1}{3}$ oz. cyanide, 1000 c.ft., exposure three hours, gave 100 per cent. mortality for adults and a poor result for scales. In the two last cases tomato plants were damaged, the temperature being 57° and 62° respectively. To control the infestation by these means at least three fumigations would be necessary as against two when the smaller doses with long exposures are used. The former at the present price of materials would cost about £12 per acre and the latter about £4. Provided that the houses are opened up at dawn there appears to be no more risk to the plants with the long exposure and small dose than with the short exposure and large dose, and the former should always be employed.

Toxicity of the gas. The following account of the toxicity of the gas for the insect deals only with exposures of 8–11 hours' duration.

The eggs are unaffected and the adults are rather more susceptible than the scales. A large proportion of the adults fall to the ground directly the gas reaches them and unless there is a sufficient concentration many of these recover during the following day or two and regain the plants. Whenever the mortality of the adult was total that of the scales was always more than 90 per cent., generally more than 95 per cent. and often 100 per cent., so that if all the flies are killed the effect of the fumigation is known at once to be good, if not perfect. The results obtained on heavily infested plants sleeved after the fumigations will be given briefly under the various dosages. The study of the life-history was carried on at the same time and showed that the emergences recorded were after intervals too brief for them to have been in the egg condition at the temperatures ruling at the season when the particular fumigation was done.

½ oz. cyanide per 1000 c.ft.; 4 tests; mortality for adults always total; mortality for scales in three cases total; small numbers of adults emerged on one plant beginning on the 18th day after fumigation.

⅓ oz. cyanide per 1000 c.ft.; 6 tests; mortality for adults always total; mortality for scales in three cases total and in three cases very small numbers emerged beginning on the 13th, 16th, and 17th days respectively.

¼ oz. cyanide per 1000 c.ft.; 14 tests; mortality for adults always total except in one case when three out of 500 recovered; mortality for scales in seven cases total; in three cases a single scale survived out of hundreds, the flies emerging on the 8th, 14th and 15th days respectively; in four cases emergence occurred in very small numbers (one to three a day) commencing once on the 10th, twice on the 14th, and once on the 17th days respectively.

⅕ oz. cyanide per 1000 c.ft.; 10 tests; mortality for adults always total except in two cases, 1 and 9 surviving respectively, in each case out of many hundreds; mortality for scales in six cases total; in two cases emergence occurred in small numbers after the 12th day, in one case two flies emerged on the 5th and no more to the 10th day; the remaining case was an unexplained failure, only 75 per cent. of the scales being killed.

⅙ oz. cyanide per 1000 c.ft.; 9 tests; mortality for adults total in five cases, and in the remainder over 95 per cent.; mortality for scales total in three cases; emergence in the other cases in very small numbers began on the 2nd, 5th (twice), 6th, 7th and

10th days respectively. In one case where an exact count was made after a fumigation lasting 9 hours on six tomato plants holding scales from 9 days old to mature pupae the mortality was 91 per cent. (2900 out of 3184).

The fact that the small numbers surviving these fumigations were apparently young for the most part at the time of treatment is difficult to explain as, when smaller doses of cyanide were used, it was found, as other workers have stated, that the pupae, which would give rise to adults in two or three days' time, were the most resistant forms. Series of plants were prepared as described above so that in each series one plant held scales representing one day's development from egg to adult; *i.e.* at one end of the series was a plant holding only eggs, due to begin hatching, and at the other end was one holding mature scales from which emergence of flies had just commenced. Two series were cyanided as follows: (1) 18 tomato plants representing complete development except that three plants died from stem rot and caused gaps; fumigated with $\frac{1}{8}$ oz. cyanide per 1000 c.ft., 9 hours' duration; (2) 19 bean plants representing complete development, fumigated with $\frac{1}{8}$ oz. of cyanide per 1000 c.ft.; $9\frac{1}{2}$ hours' duration. After the treatment the plants were kept for a week and a count was then made of the living or emerged scales and the dead ones, which had by this time turned brown and dried up.

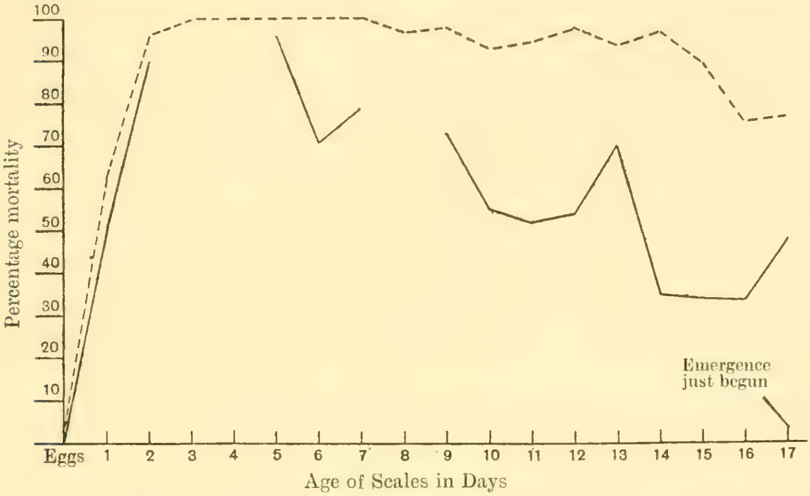


Diagram II. Showing percentage mortalities of scales of *A. vaporariorum* of various ages obtained with small doses of cyanide, long exposures. Compare with Diagram I. The gaps on the lower curve are due to the death of three plants. $\frac{1}{8}$ oz. cyanide per 1000 c.ft. $\frac{1}{7}$ oz. cyanide per 1000 c.ft.

The smallest count made on any one plant was 140 and the highest 970, while the average number dealt with was 460. The mortalities on the several days reduced to percentages are shown in Diagram II. The mortality on a tomato plant uncyanided but otherwise similarly treated was 29 dead out of a total of 900 scales, or 3 per cent., while the natural mortality on uncyanided beans was also negligible. If these two curves, and especially the lower one given by $\frac{1}{8}$ oz., are examined in relation to Diagram I which shows the proportion of the scales which would be in the different stages on each day, it will be seen that there are very suggestive drops in the mortality which correspond roughly with (1) the first moult on the sixth day after hatching; (2) the second moult on the eighth day, seen only in the upper curve; (3) the third moult on the tenth to twelfth days, and (4) the time of true pupation just before emergence begins. The mortality of the adults given by these small doses varied with $\frac{1}{7}$ oz. from 95–100 per cent. (average of five tests 98 per cent.), and in 10 tests with $\frac{1}{8}$ oz. from 88 per cent. to nearly 100 per cent., but was never total with the weaker charge.

After these experiments it appeared possible that a very good result could be obtained with two fumigations with a small charge on successive nights, and two heavily infested tomato plants were treated with $\frac{1}{8}$ oz. cyanide per 1000 c.ft. On the following morning one of them was removed from the fumigation greenhouse while the other was treated again the next night with the same charge. The mortality of the scales in all stages, on the first plant was 75·8 per cent. (805 out of 1045) and on the plant treated twice 91·8 per cent. (903 out of 1013). The combined effect of the two thus gave a less satisfactory result than would have been obtained with $\frac{1}{6}$ oz. in one fumigation.

The very small charges will clearly give a moderately good check to the pest, though they will not exterminate it, and it is at times advisable to use them on soft sappy tomato plants which for one reason or another cannot be brought into a proper condition to withstand the normal dosage.

Dosage. From these experiments and tests made in commercial houses it was concluded that in an isolated greenhouse in a moderate state of repair $\frac{1}{4}$ oz. of cyanide per 1000 c.ft. could be relied on to give practically total mortality for all stages except the eggs, if the fumigation lasted through the night. In a block of greenhouses in decent repair which communicate with one another a dose of this size is not required as the proportional leakage is less and $\frac{1}{5}$ oz. per 1000 c.ft. is a sufficient quantity, or when the houses are new and in very good repair even $\frac{1}{6}$ oz. gives almost total mortality.

The amount of cyanide which should be put into one jar depends on the width of the houses which vary in the Lea Valley from 12 to 30 feet. A good rule to follow is to so arrange the jars that the distance between two is approximately the width of the house as this arrangement gives an even distribution of the gas. Quarter ounce charges are recommended for houses up to 14 ft. wide, half ounces for houses 14–20 ft. wide, and ounces for wider ones. It is not wise to use a larger charge than this in tomato houses as the concentrated gas evolved so close to the plants is liable to cause damage.

The use of liquid hydrocyanic acid has recently been advised by Quayle(7) for the fumigation of fruit trees as an alternative to the jar method of generation, but the difficulties of distribution make this impracticable in large greenhouses.

Repetition of fumigation. The fumigation should be repeated when all the eggs have hatched but before any of the young can become adult. A reference to Table III shows that at any temperature there is an interval of a few days between these periods in heated greenhouses. The most suitable days for the second fumigation are shown in the poster "Cyaniding Tomato Houses."

Effect on plants. While the doses of cyanide mentioned above may be applied without hesitation to most greenhouse plants, very considerable caution is required in applying them to tomatoes, whether growing in pots or in borders, as this plant is particularly susceptible to damage by the gas. Hard growing wiry tomato plants resist the gas much better than soft sappy ones, but in trade nurseries the plants are usually of the latter type (Plate I, fig. 3).

The damage to which they are liable consists of burns on the foliage which may develop at once or several days after the fumigation. The damage is symmetrical on the leaflets and in moderate cases is confined to the basal half on each side of the midrib. Leaves which are fully grown are much less liable to damage than the younger ones. In mild cases the leaflets of the younger leaves crinkle up without any browning. On several occasions the only damage which has occurred has been a scorch on the underside of the petioles of two or three leaves which causes a permanent dwarfing of the leaf. The leaflets develop normally but become very crowded, while sometimes the leaf coils spirally round the main stem. The growing points and stems and trusses are only damaged by exaggerated carelessness such as fumigating in daylight or by the use of excessive doses. Faulty setting of the fruit could never be associated with fumigation.

By taking continuous measurements of the growth of very vigorous plants by means of a Farmer auxanometer it was found that when the fumigation commences a distinct check in growth occurs when doses of $\frac{1}{4}$–$\frac{1}{6}$ oz. per 1000 c.ft. are used, even if no subsequent lesions develop. After a day or two a normal rate of growth is resumed. One of these growth records is reproduced in Fig. 5. The plant from which this was taken was young, about $2\frac{1}{2}$ ft. in height and of a moderately soft nature, with the second truss setting. One pint of water was given daily. The thread was tied close behind the growing point and the pointer was reset at noon each day. The actual growth on six successive days was as follows, the fumigation lasting on the third period from 9.0 p.m. to 6.0 a.m.: (1) 1·25 mm., (2) ·9 mm., (3) ·8 mm., (4) ·3 mm., (5) ·35 mm., (6) ·95 mm. Very slight damage to the foliage followed the fumigation. A similar check in the growth of the length of the leaves also occurs though unaccompanied by any obvious damage.

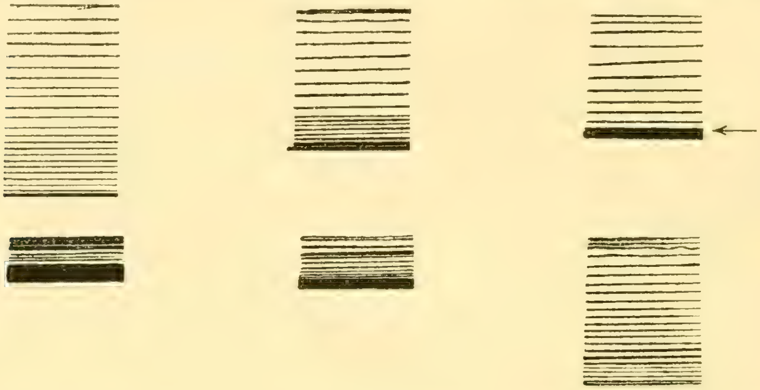


Fig. 5. Record of daily growth of tomato plant measured by Farmer auxanometer, showing check and recovery in growth after fumigation with hydrocyanic acid ($\frac{1}{4}$ oz. cyanide per 1000 c.ft., duration 9 hours). Instrument set to record double the growth. The gas was introduced on the third day at the point marked by the arrow.

A natural assumption is that the gas causes damage by entering the leaf through the stomata but this is not necessarily the case. In this connection two experiments suggested by Prof. V. H. Blackman were carried out. Two tomato plants were placed in a dark chamber 5 hours before dusk, while four similar plants were kept in the light. At dusk all the plants were placed close together and fumigated with $\frac{1}{4}$ oz. of cyanide per 1000 c.ft., duration $9\frac{1}{2}$ hours, temperature 69–60°, relative humidity 97 per cent. The experiment was repeated with a plant kept in the dark 6 hours before dusk and then fumigated with a control plant of similar nature; ¼ oz. cyanide per 1000 c.ft., duration 9½ hours, temperature 61·5–53°, relative humidity 81 per cent. The plants kept in the dark would presumably have had more opportunity to close the stomata but were in no way protected against damage thereby as in each experiment precisely similar burns developed on all the plants in each test. The second experiment consisted in cutting with scissors the laminae of the leaflets parallel to the chief lateral veins, and in cutting off the tips of some of the leaflets. Four plants were treated in this way immediately before fumigation so that the gas had free access to the raw tissue. Although moderate burns developed no damage could be associated with the cuts and the injury was much the same on cut and uncut plants. After these experiments Prof. Blackman agreed that no simple explanation of the mode of injury could be given and it is not proposed to discuss it further.

It was abundantly clear, however, that after daylight the most important factor in fumigating with this gas was the turgidity of the plant. An idea prevailed among the growers, and had found expression in a tradesman's circular, that flaccid plants were very liable to damage. For this reason flaccid and very turgid plants were repeatedly fumigated side by side and in each case the foliage of the former escaped injury while that of the latter was damaged (Plate II, figs. 4–7). On four occasions series of five to eight large tomato plants in 12-inch pots were prepared by withholding water from them on successive days till one or two were flagging and one was excessively turgid while the remainder were in intermediate conditions. On two occasions the test consisted of the fumigation of two plants only, one flaccid and one turgid in each instance. The fumigation greenhouse was heavily saturated with moisture, except in one case, and the plants were then treated with ¼ oz. cyanide per 1000 c.ft., duration 9 hours. Flaccid plants and those approaching flaccidity escaped any damage under the following conditions of temperature and relative humidity: (1) 60–54°, 97 per cent.; (2) 66·5–61°, 88 per cent.; (3) 67–59°, 67 per cent.; (4) 69–60°, 97 per cent.; (5) 58–55°, 90 per cent.; (6) 65–52°, 85 per cent. In each case turgid plants side by side with the others were badly damaged, the injury being always proportional to the turgidity. The two following experiments carried out under still more adverse conditions illustrate the same point:

(1) Two plants with four trusses set, one plant turgid and one flaccid, were fumigated with the excessive dose of ½ oz. cyanide per 1000 c.ft., duration 9 hours, temperature 64–59°, relative humidity 86 per cent. The turgid plant had its foliage

very severely burnt while that of the flaccid one was undamaged but its buds and flowers were injured.

(2) Two young plants with first truss in flower, growing in 8-inch pots, one turgid and one flaccid, were fumigated in a chamber (350 c.ft.) at the rate of ¼ oz. cyanide per 1000 c.ft., the equivalent of at least double this charge in a greenhouse; duration 2½ hours, temperature 90–72°, relative humidity 80 per cent. Even at this excessively high temperature the flaccid plant was undamaged while the top of the turgid one was killed and the whole plant very severely scorched.

Nothing in this experimental work afforded any evidence that the humidity of the atmosphere of the greenhouse was a factor which needed to be considered in the fumigation and when plants were sprayed with water immediately before the operation no damage could ever be associated with this. Previous workers have disagreed about the importance of this factor(8) and it is probable that some have failed to dissociate the moisture of the air from that of the soil, the latter being all-important. It is fortunate for the tomato grower that air humidity is not a factor as the tomato house has necessarily a very moist atmosphere when it is closed down.

It is not, of course, practicable to allow tomato plants on which the fruit is setting to flag as the crop would be thereby damaged, but the principle has a practical application in that the grower may be advised to get the roots of the plants as dry as possible without harming them. It is a common practice in the Lea Valley to drench the borders heavily before planting (in one rather exceptional case to the equivalent of a 9-inch rainfall) so that the plants can grow for two or three months without heavy watering. (This makes the plants throw deep roots.) When the houses are in this condition there is clearly no control over the turgidity of the plants. Those who grow in pots usually keep the soil very wet while the first four trusses are setting. Under these circumstances it is advisable to use only half the quantities of cyanide recommended above which will give a very fair check to the pest and prevent it from getting out of control. Later the plants in borders are watered periodically according to the character of the soil, generally once a week, and older plants in pots do not suffer if they are allowed to dry until the "pot rings hollow." The fumigation with the full amount of cyanide may be given the night before the periodical watering is due or when the soil in the pots is dry. The day after the fumigation the plants may be watered freely.

It is not possible to give any guarantee that with these doses no damage to the foliage will occur, but an assurance may be given that if all the rules are followed any injury will be negligible in proportion to that caused by an unchecked infestation of the pest.

(5) *Summary of Cyaniding.* A poster giving the essential instructions on the cyaniding of tomato houses has been issued from the Lea Valley Experimental Station. The foregoing instructions are summarised there and reference is made to several important points which are well recognised and do not require discussion.

10. SUMMARY.

The insect exhibits partial adaptation to a temperate climate, the egg and adult being resistant to considerable cold.

The wide range of its food plants is indicated.

The adults are gregarious and show marked colour reactions. The life is long and the fecundity great. Parthenogenesis occurs and only male offspring result from this; mating is the rule and produces offspring of both sexes.

The incubation period of the egg varied from 8 to 117 days according to temperature, and the duration of the scale stage from 17 to 43 days.

The occurrence of *A. sonchi* Kotinsky in England was noted.

The attacks of the pest on tomatoes mainly make it of great economic importance.

Specialisation in tomato growing to the exclusion of other crops is a useful precaution and other precautionary measures are indicated.

Fumigation is the only effective method of treating infested plants. Naphthalene and tobacco preparations give little relief. Tetrachlorethane is a good fumigant, but is too costly for trade growers.

Cyaniding is the best method of treatment. The dose of sodium cyanide varies from one-quarter to one-tenth ounce per thousand cubic feet of greenhouse space, according to the type of greenhouse and the condition of the plants. Long fumigations with these small doses are more effective than short fumigations with larger quantities.

The precautions necessary to avoid damage to the plants are given, avoiding daylight during fumigation and having the roots of the plants dry being the most important.

REFERENCES.

(1) Quaintance, A. L. and Baker, A. C. (1913–14). *U.S. Dept. Agric., Bur. Ent., Tech. Ser.* No. 27.
(2) Morrill, A. W. and Back, E. A. (1911). *U.S. Dept. Agric., Bur. Ent., Bull.* No. 92.
(3) Hargreaves, E. (1915). *Ann. App. Biol.* i, Nos. 3 and 4.
(4) Williams, C. B. (1917). *Journ. Genet.* vi, No. 4.
(5) Schrader, F. (1920). *Journ. Morph.* xxxiv, pp. 267–305.
(6) Sasscer, E. R. and Borden, A. D. (1917). *U.S. Dept. Agric., Farmers' Bull.* 880.
(7) Quayle, H. J. (1919). *Univ. California Public., Bull.* 308.
(8) Schoene, W. J. (1913). *New York Agric. Exp. Stat., Bull.* 30.

EXPLANATION OF PLATES I AND II.

PLATE I.

Fig. 1. Underside of leaf of zonal geranium showing circles of eggs of *A. vaporariorum.*

Fig. 2. *A. sonchi* Kotinsky, on *Sonchus oleraceus* in a Lea Valley tomato house.

Fig. 3. Three tomato plants cyanided side by side and photographed a fortnight after the fumigation. The hard plant was not damaged while the two soft plants show a severe scorch and crinkle of the foliage, the damaged leaves continuing to function partially. The plants are growing away from the damage.

PLATE II.

Two tomato plants photographed immediately before (Figs. 4 and 6) and a week after (Figs. 5 and 7) cyaniding together, $\frac{1}{4}$ oz. cyanide per 1000 c.ft., duration 9 hours, temperature 60–54° F., relative humidity 97 per cent. Fig. 4 represents a plant well watered and turgid, and the severe damage it received from the gas is shown in Fig. 5. Fig. 6 represents a plant which was not flaccid but required water, and Fig. 7 shows that it was undamaged.

(*Received July 27th*, 1921.)

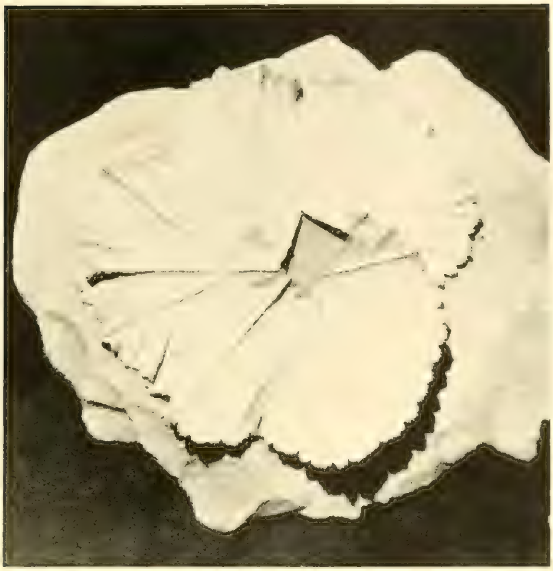


Fig. 1.

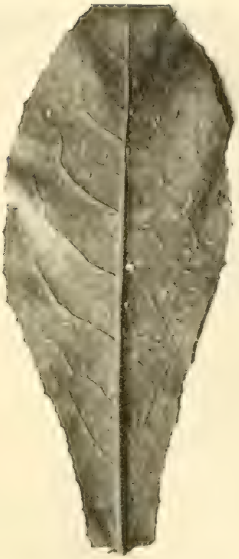


Fig. 2.

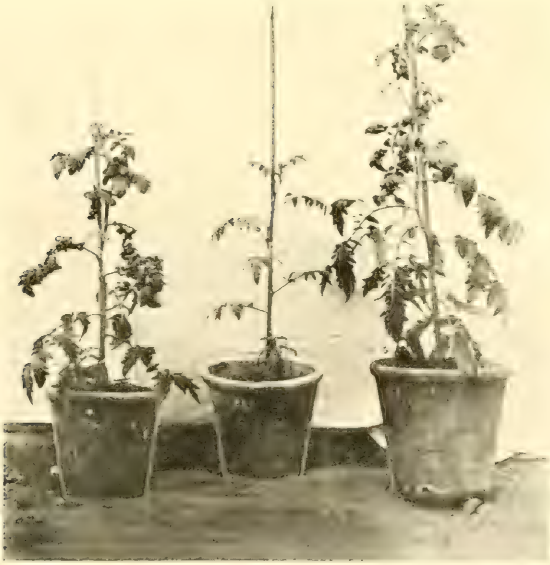


Fig. 3.

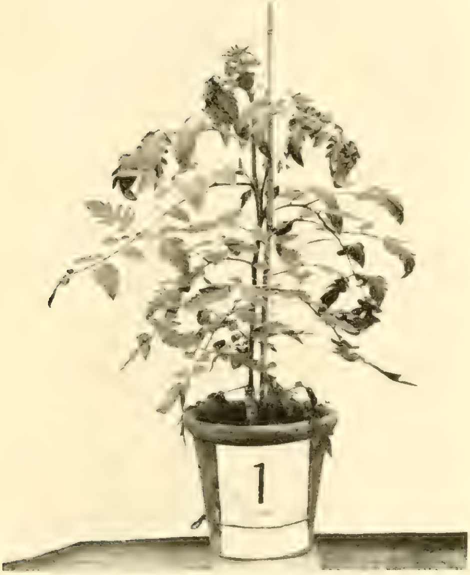


Fig. 4.

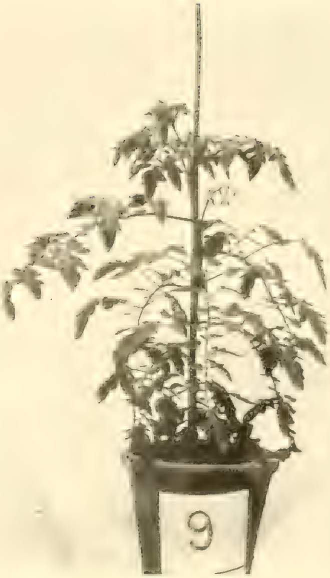


Fig. 6.

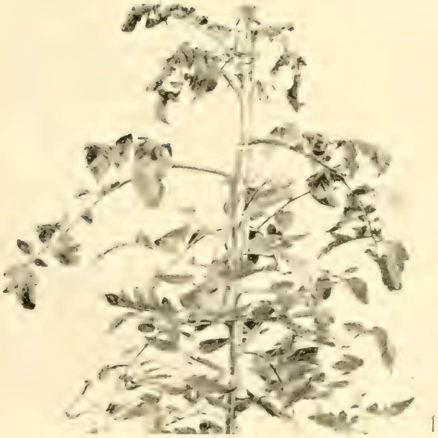


Fig. 5.

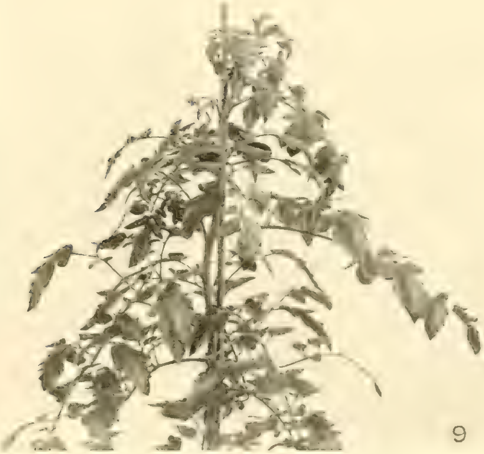


Fig. 7.

OBSERVATIONS ON THE ENSHEATHED LARVAE OF SOME PARASITIC NEMATODES.

By T. GOODEY, D.Sc.,
Helminthological Department, London School of Tropical Medicine.

(With 1 Text-figure.)

CONTENTS.

	PAGE		PAGE
Introduction	33	Resistance to desiccation and effect of plasmolysing solutions	43
Material	34	Nature of the sheath	45
Cultivation of the larvae	34	Summary	46
Attempts at skin infection	36	References	47
Effects of temperature	42		

INTRODUCTION.

At the suggestion of Professor R. T. Leiper I took up the investigation of the larvae of certain parasitic nematodes. *Graphidium strigosum* and *Trichostrongylus retortaeformis*, occurring in the alimentary canal of the rabbit, were selected because the host is a convenient laboratory animal and because the parasites are of some economic importance in causing disease amongst wild rabbits. Moreover, both worms belong to the order of Trichostrongylidae of which many members are parasitic in the alimentary tract of cattle, sheep and other domesticated animals.

One of the chief objects of the research was to discover whether the ensheathed larvae of these two species can infect through the skin in a like manner to the ensheathed larvae of *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis* and some others. The work on *N. americanus* recorded in the following pages arose from the necessity of conducting experiments with larvae known to be skin penetrators. With regard to the location of the two rabbit parasites, *G. strigosum* occurs only in the stomach, whilst *T. retortaeformis* is found mostly in the small intestine but may occasionally occur in the stomach also. The former is reported only from rodents, but the latter has been found in sheep and goats in addition to rodents.

The adult females of all three species produce eggs which pass out in the faeces of the host.

MATERIAL.

Through the kindness of Dr C. F. Druitt of Alvaston, Derby, I was able to obtain a supply of material for this research. Dr Druitt has the shooting rights of some fields where the rabbits were noticed to be suffering from certain wasting conditions towards the end of 1920. One or two specimens of these diseased rabbits were sent to this department and were found to contain large numbers of the two parasites in question.

In the early part of this year, 1921, I got into touch with Dr Druitt and he very kindly supplied me with rabbit droppings from the same areas where the wasted rabbits had occurred. At another time he sent three live rabbits, two of which turned out to be heavily infected with *G. strigosum* and *T. retortaeformis*, and furnished a good supply of droppings containing eggs, until they died after being in captivity for a short time. On opening these rabbits large numbers of both parasites were found.

I should like to express my best thanks to Dr Druitt for the interest he has taken in the work and for the great assistance he has given in providing material.

The larvae of *N. americanus* were obtained in cultures from the faeces of one of the patients in the Hospital for Tropical Diseases.

CULTIVATION OF THE LARVAE.

By teasing out a few droppings from an infected rabbit in water it was easy to find the eggs of both *G. strigosum* and *T. retortaeformis*. In the case of mixed collections of droppings from the infected area, which have been used in this work, it was possible to recognise the eggs of both worms. Identification was an easy matter also, in that a good supply of adult worms was available, and these furnished the necessary eggs for comparison and measurement.

A number of rabbit droppings were broken down in distilled water until a fairly thin mixture was obtained. This was put into a Petri dish in a shallow layer. The lid of the dish was provided with a layer of clean blotting-paper which was kept moist, and afterwards the dish was put into the incubator at 22° C.

Numerous rhabditiform larvae developed in this culture, but became quiescent on the bottom of the dish after about two days and failed to develop further. This was probably due to the presence of toxic substances in the culture, so recourse was had to Looss's recommendation of the use of animal charcoal. The next cultures were therefore put up with a liberal admixture of this substance with the teased-up droppings so that a moist, not sloppy, medium was finally obtained. This was put into Petri dishes, a shallow layer in each, and the lid of each dish was provided, as before, with clean, moist blotting-paper. The dishes were incubated at 22° C.

From these cultures large numbers of ensheathed larvae of both *G. strigosum* and *T. retortaeformis* were obtained in the course of six days.

The development of the larvae is in every way similar to that of *Ancylostoma duodenale* described by Looss (1) and of *Haemonchus contortus* described by Veglia (9).

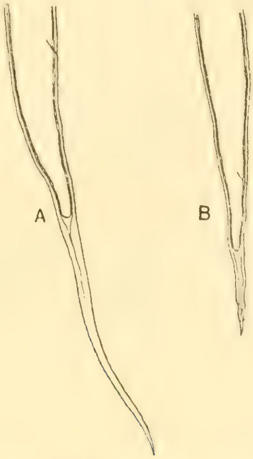


Fig. 1. Tails of ensheathed larvae of A. *Graphidium strigosum*, B. *Trichostrongylus retortaeformis*. ×350.

On the hatching of the egg a typical rhabditiform larva is produced, having a buccal cavity, the walls of which appear under the microscope as two refractive rods followed by two dots. This is followed by the oesophagus which, for the greater part of its length, is somewhat cigar-shaped, and is then constricted into a much narrower portion, and finally swells out into a bulb within which can be seen the characteristic Y-shaped lining. The intestine succeeds the bulb and presents a wavy outline amidst the granular contents of the intestinal cells and terminates in the anus. After the first ecdysis the larva grows and the oesophagus loses its original appearance, becoming longer and without such pronounced demarcation into distinct regions. The posterior bulbous portion seems to become elongated and at the same time flattened. The cells of the intestine become densely crowded with reserve food granules which sharply set off this region from the rest of the body.

Finally, at the end of this stage of growth, the larva becomes ensheathed by the replacement of its cuticle by a new one underneath. The mouth and anal apertures close up, and at the same time the enclosed larva shrinks a little in size so that it becomes separated from the old cuticle which encases it as a completely closed sheath. It then wanders upwards from its surrounding medium.

The ensheathed larvae of *G. strigosum* and *T. retortaeformis* are similar to each other in all essential structures. The former have much longer tails than the latter, and by this means can be easily recognised in a mixture of the two kinds (Fig. 1).

ATTEMPTS AT SKIN INFECTION.

(1) A large number of ensheathed larvae were collected from the culture dish lids containing moist blotting-paper by pouring distilled water on to the latter and allowing this to stand for a short time. These were concentrated in a small quantity of water by centrifugalising. A drop of this liquid was placed on the skin of a young rat (10 days old), in the inguinal region, and allowed to remain there for 20 minutes, the animal being held in position during this time. The water was not allowed to dry up, a drop being added to replace that lost by evaporation. It was then killed and the portion of skin was dissected out and fixed in 10 per cent. formalin. This was embedded in paraffin and sectionised, but the sections showed no sign of skin infection.

(2) The day following this experiment a large number of larvae in water were placed in the bottom of a glass tube, and into the liquid the hind-quarters of another young rat were immersed. The larvae could be seen moving actively in the liquid when examined through a hand lens. The animal was kept in the tube for one hour, during 20 minutes of which the tube was in the 37° C. incubator. After this the rat was chloroformed and the skin from the tail and both hind feet was fixed in 10 per cent. formalin. It was noticed that the larvae left in the tube after taking out the rat were very sluggish in movement and many were practically quiescent. I associated this with the fact that they had been put into the 37° C. incubator and inferred that the increase in temperature had checked their motility. Some of the larvae were placed on a slide in a drop of water and covered with a coverslip; they then became quite active again. This was no doubt due to the return to normal room-temperature as many subsequent observations proved.

Examination of the sections from the tail and one of the feet failed to reveal any sign of skin infection.

(3) A young mouse (body about 1 inch long) was chloroformed and the skin from the abdomen and flanks removed. This skin was stretched and pinned over a hole about $\frac{1}{2}$ inch in diameter in the centre of a piece of sheet cork $\frac{3}{16}$ inch in thickness. The cork was then floated on the surface of some *N* saline which had previously been warmed up to 37° C. and placed in a glass jar 3 inches high by $2\frac{1}{4}$ inches in diameter, having a well-fitting stopper. The cork floated near the top of the jar and care was taken that the warm saline came into contact with the underside of the skin by first allowing the bubble of air to escape from between the skin and the cork. The jar could be placed on the stage of the binocular dissecting microscope and the surface of the skin easily examined.

A drop of water containing a large number of active larvae was placed on the skin. Immediate examination showed them to be actively moving in the drop. The jar was placed in the incubator at 37° C. and examined every quarter of an hour during a period of one and a half hours. During this time some of the larvae could be seen moving very sluggishly amongst some threads of blotting-paper in the drop, whilst others moved more actively. As far as could be seen there was no down-boring motion exhibited by the larvae and no sign of skin penetration. At the end of an hour and a half the bulk of the supernatant water of the drop was drawn off by means of a capillary pipette, and a drop of fresh white of egg was placed on the skin. Examination under the microscope showed the larvae moving in this. The albumen was then coagulated by dropping hot 90 per cent. alcohol on to it from a pipette. This was done so as to fix in position the larvae which had been added with the original drop of water. The skin was then immersed in 10 per cent. formalin for fixation. It was later on treated in the usual way and embedded in paraffin for sectionising. Examination of the stained sections failed to reveal any sign of skin infection, though many slides showed sections of larvae between the coagulated albumen and the epidermis.

I realised that in the above described experiments I had, quite possibly, not brought about the conditions requisite for the larvae to penetrate the skin, *i.e.* conditions under which known skin penetrators such as the larvae of *Ancylostoma duodenale* or *Necator americanus* would act. I therefore determined to obtain a culture of the eggs of one of these forms and rear a number of the ensheathed larvae with a view to finding out the exact experimental conditions required by these for skin penetration.

In due course a stool was obtained containing a large number of adult *N. americanus* together with their eggs. Cultures of the faecal matter were put up with animal charcoal in large Petri dishes the lids of which were provided with a layer of blotting-paper, which was always kept moist. After eight days a good supply of ensheathed larvae was collected from the lids by washing the blotting-paper with water. The washings were centrifugalised and the larvae concentrated into a small bulk of water.

A young rat, three days old, was chloroformed and the skin from the abdomen and flanks was removed. This was stretched over a hole in a piece of sheet cork and pinned in position. The cork was then floated on the surface of *N* saline warmed to 37° C. contained in a glass jar as already described.

Examination of the drop under the microscope showed the larvae in very active movement with their anterior ends pressing downwards on to the skin as though trying to get into it. The jar was closed by its stopper and placed in the incubator at 37° C. At frequent intervals it was taken out and after removal of the stopper the drop was examined under the microscope. Always the larvae were seen to be very actively wriggling in a downward direction, but at the end of two hours there was no sign of any of them having escaped from their sheaths and penetrated the epidermis. I did not understand the reason for this as I was under the impression that I had brought about the requisite conditions for skin penetration. However, I put the jar with the cork and its attached skin back into the incubator, but with the stopper left out. On examining the preparation under the microscope the following morning I found that the drop of water had evaporated and that on the surface of the skin, within which many larvae could be seen, there were several empty sheaths. From this I inferred that evaporation of the water containing the larvae was necessary before they could leave their sheaths and penetrate the skin. At all events it seemed probable that there was some mechanical necessity for a shallow drop or even a film of water rather than a globule for the larvae to act in. In a deep globular drop, although they could be seen actively wriggling downwards on to the skin, they seemed to lack the necessary purchase of pressure upwards against a resistant surface to enable them to leave their sheaths and penetrate the skin[1].

---

[1] Looss's description ((4), p. 431) of his repetition of Herman's experiment on the effect of methyl-green stain on ensheathed *Ancylostoma* larvae bears on this point. He found that if the drop containing the larvae and the stain was not covered with a coverslip

The day following this experiment two more were set up. In one I placed a drop containing many active larvae on the surface of a piece of skin taken from a young rat's abdomen as on the previous day and under the same experimental conditions. The jar with its floating raft of cork carrying the skin stretched over the hole with the saline in contact with the lower surface of the skin was placed in the incubator at 37° C. but the stopper was out of the jar. Examination showed the larvae in active movement, wriggling downward, their anterior ends pressing against the surface of the skin. Several examinations were made during the course of practically two hours and each time the larvae could be seen actively in motion. The last time the preparation was examined, *i.e.* after 1 hour and 55 minutes, it was found that the drop had completely disappeared and, owing to the conditions of illumination, it was difficult to make out the details of the surface of the skin. I therefore placed a drop of clean distilled water on the surface of the skin. There could now be seen a large number of empty sheaths and one or two freely moving larvae. Practically all the larvae, however, had left their sheaths and penetrated the skin, where they could be seen moving slightly when the preparation was suitably illuminated. The drop of distilled water was removed by means of a pipette, and when examined later on was found to be rich in empty sheaths. The skin containing the larvae within it was fixed in hot 70 per cent. alcohol and the next day a portion of it was divided into two layers, for the epidermis and the immediately subjacent layers separated very easily from the dermis, and the upper epidermal layer was cleared in lactophenol and mounted whole. In this way a most interesting slide was obtained showing empty sheaths on the actual surface of the skin whilst just below, embedded in the epidermis, could be seen numerous larvae which had penetrated the skin.

In the other experiment, which ran concurrently with that just described, I used a piece of skin from the back of a young rat. The skin was pinned on to a sheet of cork as before, and instead of putting the drop of water containing the larvae directly on the skin, I placed on the latter a small piece of clean blotting-paper. This was done to imitate in a miniature way Looss's ((4), p. 519) experiments, in which he applied a pad of sacking or gauze to a dog's skin and then put the active

---

the larvae could not get out of their sheaths. They required the presence of a downward pressure of the coverslip before they could obtain the necessary purchase to break the anterior end of the sheath open and then creep out. I repeated this experiment with *Necator* larvae and obtained the same result.

Ancylostoma larvae in suspension on the surface of the pad, giving them two hours in which to bore through the material and get into the skin underneath. I ensured that the blotting paper had good contact with the skin below, and then applied a suspension of active larvae to its upper surface. Immediate examination under the microscope showed the larvae actively wriggling on the blotting-paper. At the end of two hours no larvae were to be seen, and the blotting-paper was removed and put into a drop of distilled water for subsequent examination. The skin just beneath the blotting-paper showed two or three larvae moving on it, but it was impossible to see into the skin because it was too dense and rather pigmented. The preparation was placed entire into hot 70 per cent. alcohol, and on the following day the epidermis was split from the dermis and cleared in lactophenol. When mounted on a slide it was found that numerous larvae were embedded in it.

The water in which the pad of blotting-paper was placed was found to contain numerous empty sheaths. No active larvae emerged from it, thus showing that all had passed through it to the skin.

I have described these experiments in some detail because they reveal a convenient and easily manageable method of experimentation for skin infection work.

I next proceeded to use this method with the ensheathed larvae of *G. strigosum* and *T. retortaeformis*. A young rat, seven days old, was secured and chloroformed. The skin was removed from the abdomen and flanks and was found to be very soft and tender. It was stretched on a sheet of cork and pinned over the hole in the manner already described, and then the cork was floated on normal saline at 37° C. A drop containing numerous active larvae was placed on the surface of the skin, and it was at once evident, on examining the drop under the microscope, that the reaction of these larvae to the temperature of the saline, 37° C., was quite different from that of the *Necator* larvae. The latter executed lively downwardly directed movements as though trying to get into the skin, and were decidedly more motile at 37° C. than at laboratory temperature. The *G. strigosum* and *T. retortaeformis* larvae, on the other hand, very quickly became sluggish in their movements at the higher temperature. In fact they seemed to be upset and incommoded by the new conditions and made no downwardly directed movements. The stopper was left out of the jar and the latter was put into the incubator at 37° C. It was taken out at various intervals and the drop, which gradually evaporated, was examined under the microscope. It could then be seen that most of the larvae aggregated to one side of the drop and coiled up, watchspring-wise, and remained quiescent. At the end of two hours only one or two could be seen moving on the surface of the skin, and these rather slowly and aimlessly.

The jar was left in the incubator overnight, and in the morning was put out on the laboratory table and left until it reached room temperature. It was then examined and the larvae were seen to be slowly moving about. Replaced in the incubator and left overnight again, it was examined on the following morning, when it was seen that most of the larvae were still crawling about and had evidently become accustomed to the higher temperature. The skin was split into two layers, as had been done in the *N. americanus* preparation, and examined under the microscope. It was then easy to see that the larvae were still ensheathed and had made no attempt to penetrate the epidermis after contact with it for 48 hours.

The foregoing experiments give no evidence that the ensheathed larvae of *G. strigosum* and *T. retortaeformis* are capable of penetrating through the skin.

Veglia ((9) p. 424) in his paper on *Haemonchus contortus* gives an account of his attempts to get the ensheathed larvae of this worm to infect through the skin. His final test of whether infection had taken place was to search for the adult worms in the intestine or the presence of eggs in the faeces. In one case larvae were injected under the skin, but though these remained alive for a short time they did not set up an infection. In all cases he failed to secure infection through the skin, so that my experiments fully support his negative results.

Theiler and Robertson ((8) p. 321) placed large numbers of ensheathed larvae of *Trichostrongylus douglassi* on the skin of an ostrich to test the possibility of skin infection. The bird used was kept under close observation for eight months but at no time were the faeces found to contain eggs.

Brumpt (2), making use of human umbilical cord, though the method of experimentation is not given, includes *Trichostrongylus* (*retortaeformis*?) (his query, not mine) from the rabbit in his list of larval forms which he says penetrate the cord tissue. The paragraph is as follows: "En nous servant du cordon ombilical humain, nous avons pu constater qu'il attire et se laisse pénétrer par les espèces suivantes: *Necator americanus*, *Strongyloides papillosus* du Mouton et du Lapin, *S. stercoralis*, *S. suis*, *S. vituli*, *S. sp.* d'un Macaque, *S. sp.* d'un Cercopithèque, *S. equinus* et *S. vulgaris* du Cheval, *Characostomum longemucronatum* du Porc, *Trichostrongylus* (*retortaeformis*?) du Lapin et par une larve d'un parasite du Mouton (*Chabertia*?)."

He meets the obvious criticism that these are not cases of skin infection by the following: "On pourra nous objecter que la faculté présentée par les larves infectieuses de certains Nématodes de pénétrer dans le cordon ombilical ne prouve pas qu'elles soient susceptibles de traverser la peau."

In view of my own results detailed above I have no hesitation in claiming that the ensheathed larvae of *T. retortaeformis* from the rabbit can be ruled out of the list of skin penetrators.

EFFECTS OF TEMPERATURE.

The behaviour of ensheathed larvae of *G. strigosum* and *T. retortaeformis* was so markedly different from that of *N. americanus* when applied to the skin at 37° C. that I determined to test the matter further.

For this purpose I used an electric warm stage in which a slide carrying a drop containing larvae can be placed and the temperature gradually raised from room temperature to 37° C. In this way I was able to watch the reaction of the larvae to the rise in temperature and to determine fairly well the optimum temperature for greatest activity. The result is shown in the accompanying table.

Graphidium strigosum and Trichostrongylus retortaeformis.

Temperature	Remarks
22–23° C.	Larvae showing good motility
23–24	Very active
24–25	Very active at edge of drop
25–26	1 or 2 showing coiling movement, rest actively motile.
26–27	A few showing sharp spasmodic backward and forward bending, rest wriggling actively
27–28	A few coiling and uncoiling, rest actively motile
28–30	Those coiled remained coiled longer, about half coiled, rest active
30–31	More than half coiled
31–32	A few moving actively, rest coiled and remaining so, 1 or 2 straight and motionless except for an occasional movement of one end
34	1 or 2 active, the rest coiled or straight and motionless
35·5	Only one moving, rest as at 34° C.
36·6	No movement

From the above it can be seen that the optimum temperature for greatest activity is between 22° and 25° C. At 25° C. coiling begins to take place and by the time normal body temperature is reached practically all motility has ceased.

This is a curious fact and not easy to understand when one remembers that the larvae require for their further development to get into the alimentary tract of the host where, of course, they will be permanently at 37° C.

They must, within quite a short period after ingestion, become accustomed to their new temperature and surroundings and resume fairly active motility in order to escape from their sheaths. It is possible that they require the specific stimulus of contact with their final environment, the stomach and intestinal walls respectively for *G. strigosum* and *T. retortaeformis*, for their emergence and further growth, though Veglia ((9) p. 426) has shown that the larvae of *Haemonchus contortus*, when taken by lambs with grass, can escape whilst in the mouth.

As bearing on this point I would refer to the observation recorded above, p. 40, in which I mention having found the larvae of *G. strigosum* and *T. retortaeformis* active after being in contact with the skin for nearly 48 hours at 37° C.

RESISTANCE TO DESICCATION AND EFFECT OF PLASMOLYSING SOLUTIONS.

Intimately associated with the power possessed by the larvae of *Necator* and *Ancylostoma* to penetrate skin is, I think, their lack of power to withstand desiccation. Looss ((4) p. 398) deals with the latter at length and says that "*Ancylostoma* larvae can remain alive on a surface which is becoming dry (and to which they adhere) so long as the envelopes surrounding them (the 'cysts' or 'sheaths') retain moisture within them. As soon, however, as this moisture begins to evaporate the bodies contract (generally in a longitudinal direction) and shrivel."

It is a very simple matter to allow a drop containing ensheathed larvae of *N. americanus* to evaporate gradually from a slide and in the course of my work I have performed the experiment a few times. It is difficult after the bulk of the water has dried up to make out the structure of the larvae, but it is very evident that they quickly feel the effects of desiccation even at room temperature, for if a drop of water is added to the slide after a few minutes, they fail to revive and resume active motion. None revive if the slide is allowed to remain dry for 15 minutes. It seems a natural inference to draw therefore that *Necator* and *Ancylostoma* larvae seek the protection afforded by penetration into skin because if they remained outside and became dry they would perish.

The ensheathed larvae of *G. strigosum* and *T. retortaeformis* on the other hand can withstand desiccation in the air for a time and when remoistened can revive and resume normal activity. Prolonged desiccation at high temperature and the action of direct sunlight are inimical to them as Veglia ((9) pp. 390 *et seq.*) has shown in the case of *Haemonchus* *contortus*, but at ordinary room temperatures they can withstand air desiccation for a few days and revive on the addition of water.

Doubtless also the habit of coiling up watchspring-wise on the advent of desiccation and further of the tendency to congregate together as drying-up proceeds constitute additional safeguards to enable the larvae to withstand the adverse effects of desiccation. It is noticeable that when *N. americanus* larvae are dried on a slide they do not coil up after the manner of *G. strigosum*, *T. retortaeformis* and *H. contortus*.

The comparative rapidity with which *N. americanus* larvae can be killed, *i.e.* by a few minutes' exposure to dry conditions, points to the sheath being very permeable to water vapour and to the contained larvae being very easily injured by withdrawal of water from its tissues. I therefore carried out a series of experiments to test this, comparing it with *G. strigosum* and *T. retortaeformis* larvae at the same time. For this purpose I made use of solutions of common salt of different strengths brought into contact with the larvae, from 15–20 in number in each case, in shallow glass capsules, and noted the effect on the organisms through the microscope. The salt solutions—5 per cent., 10 per cent., 15 per cent., and concentrated—acted by withdrawing water through the sheaths from within outwards, and had the effect of bringing about a gradual cessation of movement and finally plasmolysed the contained larvae, causing vacuolations within them.

Records of the action were taken every five minutes and at the end of each test the power of revival was tested by transferring the larvae, after first washing them in a fair bulk of distilled water, to a capsule containing more distilled water.

Five per cent. saline causes *N. americanus* larvae to slow down their movements in 20 minutes, and at 35 minutes all are quiescent or quiet. *G. strigosum* and *T. retortaeformis*, on the other hand, remain normal in movement for 20 minutes, one or two began to coil at 25 minutes, and a few remained active even for 90 minutes. On transferring to distilled water after 2 hours in the saline, only five *N. americanus* larvae showed signs of revival and this not very complete, whilst the *G. strigosum* and *T. retortaeformis* larvae all revived and swam about well.

Ten per cent. saline. *G. strigosum* and *T. retortaeformis* larvae withstand the plasmolysing action of this strength longer than the *N. americanus* larvae. They also revived in distilled water to a much greater extent than *N. americanus* larvae.

Fifteen per cent. saline. As before in the lower percentages the *G. strigosum* and *T. retortaeformis* withstood the action and remained capable of motility longer than the *N. americanus* larvae, and none of them revived in water. I performed two tests for power of revival for *G. strigosum* and *T. retortaeformis* larvae. In the first case I transferred them to water after 2¼ hours, *i.e.* after all motility had ceased, and then two or three revived after 2 hours. In the second test I transferred them to distilled water after 20 minutes in saline, *i.e.* after the period required to bring all movement to an end in *N. americanus* larvae. In this case all the larvae revived.

Saturated solution of saline. This causes cessation of movement very rapidly in all cases, and after transference to distilled water no *N. americanus* revived, but two of the others showed signs of movement.

It is clear from the above that *G. strigosum* and *T. retortaeformis* larvae are more resistant to the action of plasmolysing agents, and after the action of such are more capable of revival than the larvae of *N. americanus.*

I conclude from these results that the sheath in the case of *N. americanus* larva is more permeable than that of *G. strigosum* and *T. retortaeformis* and that the larva within is more easily injured by the withdrawal of water from its tissues than in the case of the other two organisms.

NATURE OF THE SHEATH.

It has already been pointed out that the sheath of all ensheathed larvae is produced by the replacement of the cuticle by the development of a new one underneath. It is well known too that the cuticle of all nematodes is composed of a very resistant substance capable of holding up most fixing agents and rendering the staining of these organisms a very difficult business. I decided therefore to carry out a few tests to obtain if possible a little more information as to the nature and properties of the sheath both in *N. americanus*, *G. strigosum* and *T. retortaeformis* larvae.

Martin ((5) p. 101) quotes the results obtained by Lambinet and others with the ensheathed larvae of *Ancylostoma*. Lambinet found that corrosive sublimate 0·2 per cent. does not kill them, that 3 per cent. phenosalyl arrests their movements after 1½–2 hours; Fernbach's liquor diluted 1 in 10 does not immobilise them after an hour; the pure liquor acts in ¼ hour. Five per cent. sulphuric acid kills after ¾ hour; a saturated solution of sodium bicarbonate does not kill after 2 hours, neither does Eau de Javelle after 1 hour. Camphorated petrol seems to stimulate their vitality; 3 per cent. lysol kills in 1 hour. Chloroform, ammonia and carbon disulphide all kill within 24 hours; formol vapour does not kill after this length of time. Thirty per cent. saline and pure glycerine produce strong plasmolysis and kill the larvae.

Looss ((1) p. 439) quotes the results of Leichtenstern, Lambinet, Breton and Boycott, who all tested the resistance of ensheathed *Ancylostoma* larvae to the action of gastric juice, and found that the sheaths were not affected in any way by the peptic ferment.

These results show that the sheath is composed of a very resistant substance and Martin speaks of it as *chitinous* in character.

In my experiments I found that the sheaths of *N. americanus*, *G. strigosum* and *T. retortaeformis* are insoluble in the following reagents: water, alcohol, xylol, chloroform, phenol, lactophenol, formol, glycerine.

The sheaths remain unaffected even after several days' immersion in solutions of pepsin and trypsin, though the larvae within are ultimately killed and show signs of disintegration.

The sheaths are soluble in concentrated hydrochloric acid. Those of *N. americanus* become dissolved in the course of 1½ hours at room temperature, whilst the sheaths of *G. strigosum* and *T. retortaeformis* resist the action for about 2½ hours. It is of interest to note that chitin is soluble in concentrated hydrochloric acid yielding glucosamine.

Caustic soda, 5 per cent. solution, dissolves the sheaths and the enclosed larvae at 37° C. when left in the incubator overnight, whilst a 15 per cent. solution dissolves the sheaths of all three kinds within 1¼ hours.

The action of this alkali shows that the sheath substance is not real chitin since the latter is prepared from insects, etc., by the prolonged action of alkalis and repeated washings in water.

The sheaths stain easily and uniformly with 1 per cent. solution of methyl-green and fuchsin. I found that *N. americanus* larvae came out of their sheaths, as found by Herman and confirmed by Looss, when the drop containing the larvae and the stain is covered with a coverslip. *G. strigosum* and *T. retortaeformis* larvae did not exsheath. The sheaths also stain a little with picric acid solutions.

These results show that the sheaths are composed of some substance of a resistant nature, but not so resistant as true chitin obtained from various Arthopoda, Arachnida, Mollusca and Polyzoa, since they dissolve quite readily in 5 per cent. caustic soda.

SUMMARY.

1. The eggs of *Graphidium strigosum* and *Trichostrongylus retortaeformis* give rise, under suitable cultural conditions, to larvae which finally become ensheathed and wander from the culture medium.

2. A new and easily manipulated method of experimentation for skin infection work is described, in which skin from a young freshly killed animal, rat or mouse, is stretched over a hole in a piece of sheet-cork and pinned in position. The cork is floated on *N* saline at 37° C. and care is taken to ensure that the saline comes into contact with the underside of the skin. A drop containing the larvae to be tested is placed on the upper surface of the skin. The saline is contained within a suitable glass jar and the whole can be placed on the stage of a binocular dissecting microscope and the surface of the skin examined at any moment.

3. By this method it was found that ensheathed larvae of *Necator americanus* leave their sheaths and penetrate the skin when the drop of water containing them is allowed to evaporate and become sufficiently shallow to enable them to obtain a purchase against the surface of the drop. These larvae are very actively motile at 37° C.

4. Ensheathed larvae of *G. strigosum* and *T. retortaeformis* do not penetrate the skin under exactly the same experimental conditions. They are not actively motile at 37° C. but become coiled and quiescent at this temperature. Their temperature for optimum activity is shown to be between 22° and 25° C.

5. Ensheathed larvae of *N. americanus* cannot resist desiccation in air at room temperature and cannot revive on being moistened, whereas the larvae of *G. strigosum* and *T. retortaeformis* can withstand air desiccation and are easily revived on being remoistened. It is shown that the ensheathed larvae of these two species are more resistant to plasmolysing agents than the ensheathed larvae of *N. americanus*.

6. The sheath surrounding the larvae in all three species named in the foregoing paragraph is composed of a very resistant substance whose composition is not known. It is not true chitin since it is readily soluble in 5 per cent. caustic soda.

REFERENCES.

(1) Bernard, P. H. and Bauche, J. (1914). Influence du mode de pénétration cutanée ou buccale du *Stephanurus dentalus*, etc. *Annales de l'Institut Pasteur*, xxviii, pp. 450–469.

(2) Brumpt, E. (1921). Mode de pénétration de Nématodes dans l'organisme des Mammifères, histiotropisme et histiodiagnostic. *Comptes rendus des séances de la Société de Biologie*, lxxxv, pp. 203–206.

(3) Hall, M. C. (1916). *Nematode parasites of Mammals of the orders Rodentia Lagomorpha and Hydracoidea.*

(4) Looss, A. (1911). The Anatomy and Life-history of *Ancylostoma duodenale* Dub. Pt. II. *The Development in the Free State Records of the School of Medicine, Cairo,* IV. Full references given in this to all previous work.

(5) Martin, A. (1913). Recherches sur les conditions du développement embryonnaire des nématodes parasites. *Annales des Sciences Naturelles, Zoologie,* XVIII, pp. 1–151.

(6) Railliet, A. (1889). Développement expérimental du *Strongylus strigosus* (Duj.) et du *Strongylus retortaeformis* (Zeder). *Bull. Soc. Zool. de France,* pp. 375–377.

(7) Ransom, B. H. (1911). *Nematodes parasitic in the alimentary tract of Cattle, Sheep and other ruminants.*

(8) Theiler, A. and Robertson, W. (1915). Investigations into the life-history of the wire-worm of Ostriches. *3rd and 4th Reports of the Director of Veterinary Research, Pretoria,* pp. 293–345.

(9) Veglia, F. (1915). The Anatomy and Life-history of *Haemonchus contortus* Rud. *3rd and 4th Reports of the Director of Veterinary Research, Pretoria,* pp. 349–500.

(*Received December 3rd,* 1921.)

LEAF CHARACTER IN REVERTED BLACK CURRANTS

By A. H. LEES, M.A.,
*Plant Pathologist, Agric. and Hort. Research Station,
Long Ashton, Bristol.*

(With 46 Text-figures and 11 Graphs.)

It is well known that in marked cases of reversion in black currants the leaf undergoes considerable modification. It becomes relatively long in proportion to its breadth though generally of smaller surface than normal leaves. In general effect it bears a considerable resemblance to the leaf of the stinging nettle, and the disease for this reason is frequently known as "nettle leaf." Further, when beyond the youngest stages such leaves acquire a thicker texture and a darker colour and the serrations of the margin become fewer and coarser. There is indeed no great difficulty in identifying the disease in its advanced state. At the beginning of an attack, however, it is by no means so easy to identify. Growers usually put down such cases as "suspicious" or "going" and when pressed for their reasons say that they judge from the general appearance and not from any definite signs.

Such an absence of definite data is not only unsatisfactory in the field in view of the importance of propagation from absolutely sound stocks, but it limits the experimenter very much in attempting any experiments under controlled conditions. It is obviously important for him to be able to identify with certainty the initial stages and to have some means of marking the extent of the disease on a bush from year to year. Further, unless some such means be at hand he cannot follow the course of the disease during the season so as to trace any possible seasonal variations. The method to be described was arrived at by careful comparison of a normal with a reverted leaf. It has the advantage of being a numerical one and therefore independent of the general opinion of the observer as well as of the size and shape of the leaf.

If any leaf be looked at from the undersurface, it will be noticed that there are five main veins arising from one point at the extreme base of the leaf (Fig. 23). These veins run to the five main points of the leaf, *A–E*. Now if the submain veins arising from the midrib on one side and running to points on the margin (neglecting, of course, to count the main veins to *B* and *D*) be counted, it will be found that they number at least five in a normal leaf. Sometimes there are six or seven, but never less than five. In a definitely reverted leaf, however, they are less than five, three being a common number in well-developed cases (Figs. 22*a*, 28, 29) and in extreme cases they may be reduced to zero (Fig. 46, right-hand side). The second character to observe is the margin. In normal leaves (Figs. 23, 40) there are numerous fine serrations, many of which do not receive any submain branches, but are innervated from branches of a lower order. In reverted leaves the margin has comparatively few and coarse serrations (Figs. 27–29) and only a few fine serrations exist which receive veins of a lower order than submain.

These two numerical indices have been found extremely useful both in the laboratory and in the field for exact work and immediately reveal the fact that reverted leaves are sometimes large and broad (Fig. 36), small and broad (Fig. 22*a*), or entirely irregular and deformed (Fig. 31). It also shows up the fact that small and comparatively pointed leaves need not necessarily be reverted (Figs. 2, 20).

TEMPORARY REVERSION.

Using these two methods it has been possible to study certain cases of what may be properly called "temporary reversion" which have come under the writer's experience.

Case 1. This case occurred in a pot plant kept in a greenhouse. The plant was an oldish one and in poor condition. It possessed two shoots *A* and *B*, both of which were made up of two or three years' growth. The two and three year old wood was bare of buds and of the usual blackish colour common to old black currants. Each also possessed about nine inches of one year old wood covered normally with buds.

In early spring the shoot *A* was ringed just below the one year wood. The operation was performed in the usual way, a ring of tissue about three-sixteenths of an inch wide as far as the cambium being removed. This operation was performed for an object quite apart from reversion, but it produced a very interesting effect. As usual the buds below the ring, in this case dormant ones, were forced into growth, but the leaves instead of being normal were reverted as judged by the venation and margin tests. They were not particularly narrow in shape but had a generally reverted appearance. The other shoot *B* did not break from

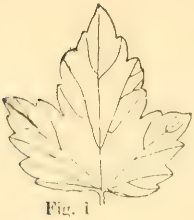


Fig. 1

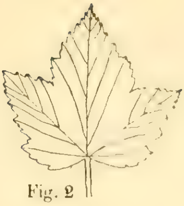


Fig. 2

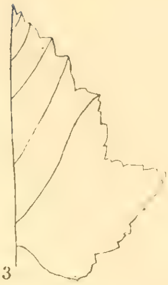


Fig. 3

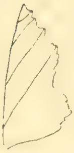


Fig. 4

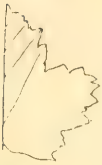


Fig. 5

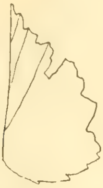


Fig. 6

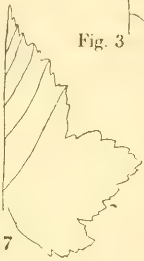


Fig. 7

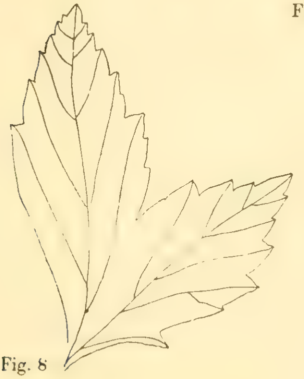


Fig. 8

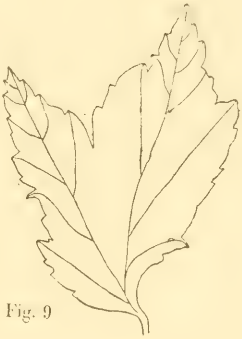


Fig. 9

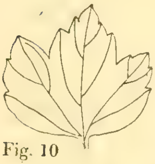


Fig. 10

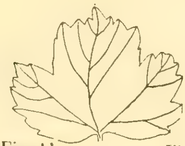


Fig. 11

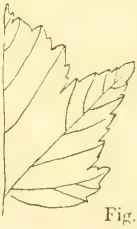


Fig. 12

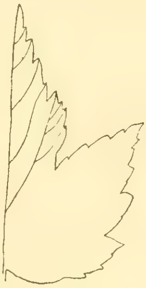


Fig. 13

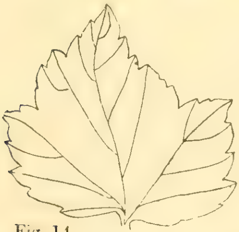


Fig. 14

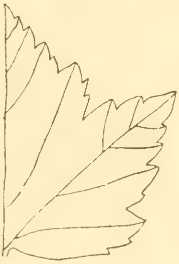


Fig. 15

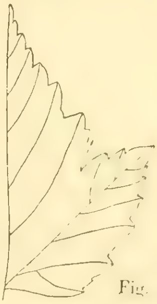


Fig. 16

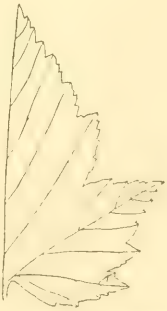


Fig. 17

Figs. 1–17. TEMPORARY REVERSION. Fig. 1. Reverted leaf from "ringed" shoot. Fig. 2. Normal leaf from wood previously bearing reverted leaves. Figs. 3–7. Case 2 of temporary reversion. Figs. 8, 9. Abnormal, deformed leaves resulting from interference to terminal bud. Shoot A, case 3. Figs. 10–13. The four basal leaves of Shoot B, case 3. Figs. 14–16. The first three basal leaves of Shoot C, case 3. **Fig. 17. 6th leaf of Shoot C, case 3.**

the older wood but produced normal though rather small leaves from the one year old wood. A few weeks after, a similar ring was made just below the one year old wood of *B*, but at first it produced no result. The whole plant at this time was suffering rather severely from the combined effects of aphis and a too hot and dry atmosphere with the result that the foliage became brown and dropped off and the plant took on a resting condition as in winter. Being of no further use apparently, it was put outside. After a few weeks the heavy summer rains caused the plant again to put out leaves, this being the second time during the season. This time, however, shoot *A* which had previously produced reverted leaves from the old wood showed perfectly normal leaves (from the buds formed in the axils of the first crop of reverted leaves). The portion above the ring had died. Shoot *B* this time produced reverted leaves from the dormant buds on the old wood in the same way that shoot *A* had done earlier in the season. The top portion of shoot *B* above the ring had also died. Outline drawings from a leaf of the second crop of shoots *B* and *A* are shown in Figs. 1 and 2. These two shoots therefore had reversed their behaviour, *A* producing first reverted leaves and then normal and *B* producing first normal and then reverted. Since they both belonged to the same plant it is clear that it could not be reverted in the ordinary disease sense.

Case 2. In order to test the effect of cutting back during the summer season a bush growing in the open had about three inches of growth cut away from every growing tip. (The original idea was to test the character of the foliage issuing from the weak lateral buds immediately below the pruning cut.) Owing to the lateness of the season very little growth occurred. This was, however, sufficient to show that the leaves produced under these circumstances were of the reverted type. Luckily, a shoot in the middle of the bush had been overlooked. This shoot had almost, though not quite, ceased growth in length and the stimulus placed upon it by removal of the active growing points from all the other leaders caused it to react in a very interesting manner. Fig. 3 shows a half outline drawing of the leaf that had just been formed before the stimulus began. It has fine submain veins and is almost normal in margin. The next leaf (Fig. 4) has lost a vein, the margin has become irregular and the leaf undersized. The next leaf (Fig. 5) is similar though somewhat larger, while in the next (Fig. 6) the veins have been reduced to three. The last in the series (Fig. 7) has regained the five veins and has become normal again in margin and outline. This therefore is clearly a case of temporary reversion.

Case 3. This case occurred in a cutting bed. When found, the single shoot arising from the cutting had divided (during the summer) into two shoots, one longer and one shorter. The reason for the division could not then be ascertained, but the original terminal growing point had absolutely disappeared and growth had been taken up by lateral buds immediately below. In Table I are found details of the important points. These are the venation of the leaf, the mites found in the axillary buds and the leaf margin and shape. The whole plant was free from mite infection at the time of examination. Characteristic temporary reversion is shown by the basal leaves of both longer and shorter shoots (*B* and *C*). In shoot *B* the two basal leaves (Figs. 10 and 11) have only three submain veins but a recovery is quickly made to five in leaf 4 (Fig. 13) and maintained to the end. Recovery of the margin is slower. Shoot *C* shows much the same transition. The first two leaves (Figs. 14 and 15) though broad are markedly reverted. The leaf venation has recovered by leaf 3 (Fig. 16), but the margin not until leaf 6 (Fig. 17).

The effects of the check to terminal growth, however, are not confined to the two shoots *B* and *C*; it has also marked influence on the undivided base of the cutting, here called shoot *A*. Normality of veins and margin is maintained up to leaf 9, namely five leaves behind the critical point. Leaf 10 was very small, deformed and of a general reverted type. Leaf 11 was unusually large but quite normal as if extra food supplies had been diverted into it. Leaves 12 (Fig. 8) and 14 (Fig. 9) were both peculiar, first in having no bud in their axils and secondly in having lost one lobe of the leaf, in one case the left lobe, in the other the right. Both were rather reverted in margin and 14 (Fig. 9) was reverted in venation. Leaf 13 like leaf 11 was normal though of extra large size.

There is evidence here of considerable disturbance to normal growth. Leaf 10, which was the first leaf to feel the effect of the killing process going on in the then terminal, apparently had its food supplies cut off so that a very deformed and small leaf resulted. The food which should have been available for the developing terminal apparently went into leaves 11–14. Such a process may be often observed in brassica plants where the terminals have gone blind; the leaves immediately below, even if cotyledons, become large and dark green. No reason for the moment can be suggested why leaves 12 and 14 lacked one lobe nor why neither had an axiliary bud. Shoots *B* and *C* each began under the same stimulus that caused the extra large top leaves of shoot *A* and both showed temporary reversion of the leaves.

Table I. *Boskoop cutting showing disappearance of terminal buds during summer. The two laterals immediately below took up the growth. Examined August 27th,* 1920.

A.

Leaves from base	Venation	Mites in axiliary buds	Margin of leaf
No. 1	Missing	0	
2	,,	0	
3	,,	0	
4	,,	0	
5	5 submain veins	0	Nearly normal
6	5 ,,	0	,,
7	5 ,,	0	,,
8	5 ,,	0	,,
9	6 ,,	0	Normal
10	Deformed	0	Very reverted
11	6 submain veins	0	Normal. Leaf very large
12	5 ,,	No bud in axil	Reverted. Left lobe lost
13	6 ,,	0	Normal. Leaf very large
14	3 + ,,	No bud in axil	Rather reverted. Right lobe lost

At this point the shoot divided into two, no trace of the original terminal being left.

B. Longer shoot.

Leaves from base	Venation	Mites in axiliary buds	Margin of leaf
No. 1	3 submain veins	0	Very reverted
2	3 ,,	0	Very reverted. Larger
3	5 ,,	0	Less reverted
4	5 ,,	0	,,
5	5 ,,	0	,,
6	6 ,,	0	Normal shape and margin
7	6 ,,	0	,, ,,
8	6 ,,	0	,, ,,
9	6 ,,	0	,, ,,
10	5 ,,	0	,, ,,
11	6 ,,	0	,, ,,
12	5 ,,	0	,, ,,
		Terminal 0	

C. Shorter shoot.

Leaves from base	Venation	Mites in axiliary buds	Margin of leaf
No. 1	2 submain veins	No bud in axil	Very reverted
2	3 ,,	0	,,
3	3 + ,,	0	Less reverted. Larger leaf
4	5 ,,	0	Slightly reverted. Almost normal shape
5	5 + ,,	0	Slightly reverted. Almost normal shape
6	6 ,,	0	Normal end of season leaf
7	6 ,,	0	,, ,,
8	6 ,,	0	,, ,,
9	6 ,,	0	,, ,,
		Terminal 0	

Case 4. This case did not come under the personal notice of the writer but was reported by a grower. The case consisted of a temporary reversion caused by the attack of Capsid bugs.

All these four cases seem to come under one general set of conditions. In the first three and probably in the fourth a considerable impetus to growth has been thrown on to weak buds. In case 1 dormant buds were stimulated by a ring above; in case 2 a bud that was just ceasing growth was suddenly urged into fresh growth by removal of all other active growing points and in case 3 weak laterals of the current season were acted on in the same manner. In the absence of exact physiological data it is impossible to make any sort of definite statement, but the conclusion that may warrantably be drawn from these cases is that the plant reacted under a special stimulus to growth.

DISEASE REVERSION.

Under this heading are grouped certain cases which have come under the writer's notice and which appear to be produced by causes other than those treated under the heading of "Temporary Reversion." They are of course similar in every way to those that appear in growers' plantations. The differences between normal and reverted leaves are perhaps most clearly brought out by a comparison of a normal and reverted shoot taking each, leaf by leaf, comparatively. Such a dual series is shown in Figs. 18 and 18*a* to 23 and 23*a*. In the two younger leaves (Figs. 18–19, 18*a*–19*a*) there are no marked differences but in the third leaves (20 and 20*a*) the blunter appearance of the lobes is already evident. In the enlargement of the lobe *D*, two points may be noticed. First the lobes are coarser, especially the apical one which is quite broad at the base in the reverted specimen and narrow in the normal one. Secondly the submain veins tend to become reduced in the reverted leaf, the top veins showing a characteristic bending round so that they nearly rejoin the submain vein instead of running to a point on the margin. These two characters are general throughout the leaf and constitute the best numerical means of ascertaining the extent of reversion. A still more advanced stage is shown in the enlargement of *D* of Figs. 21 and 21*a*.

Figs. 21–23 and 21*a*–23*a* show successive stages in loss of submain veins from the midrib; 21*a* has four and 22*a* only three. These leaves were the fourth and fifth from the apex at the time of examination and are about the same size, but in the 10th leaves from the apex (Figs. 23 and 23*a*) a marked difference in size in favour of the normal has appeared. These may be taken as fair types of the two kinds. The changes that have occurred may be summed up under three heads: (*a*) reduction of the venation system, (*b*) coarser and fewer lobes, and (*c*) reduction of leaf size in the older specimens.

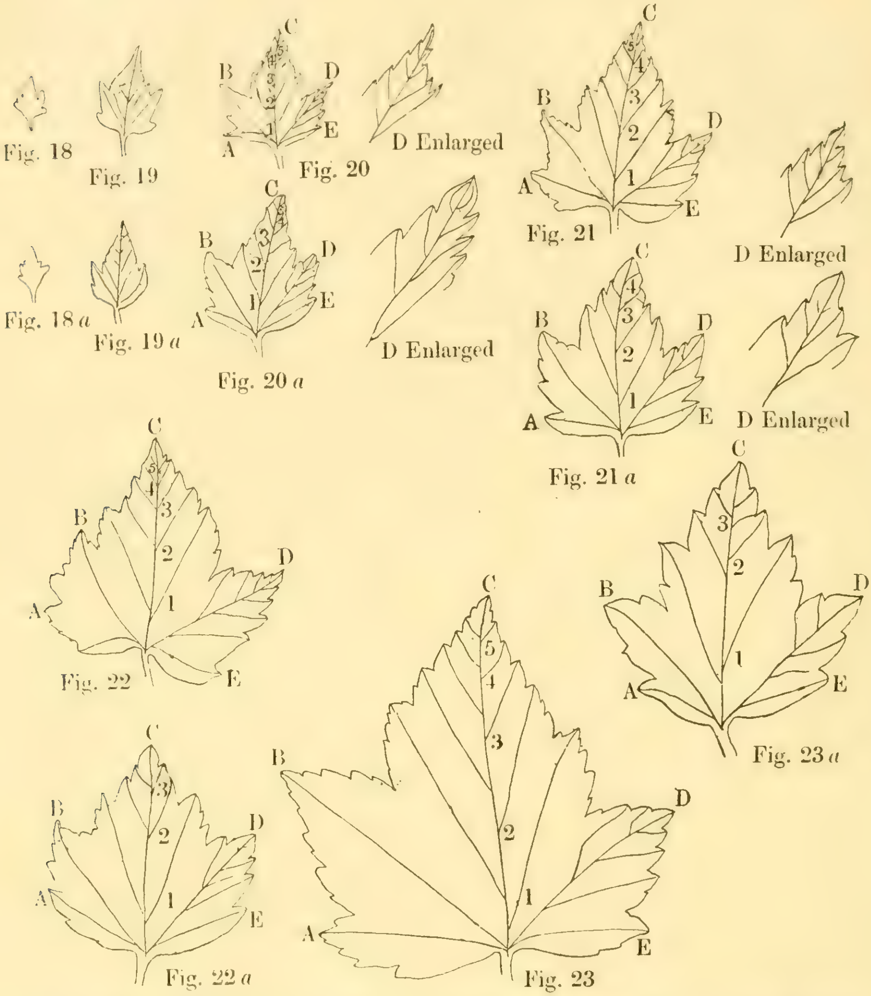
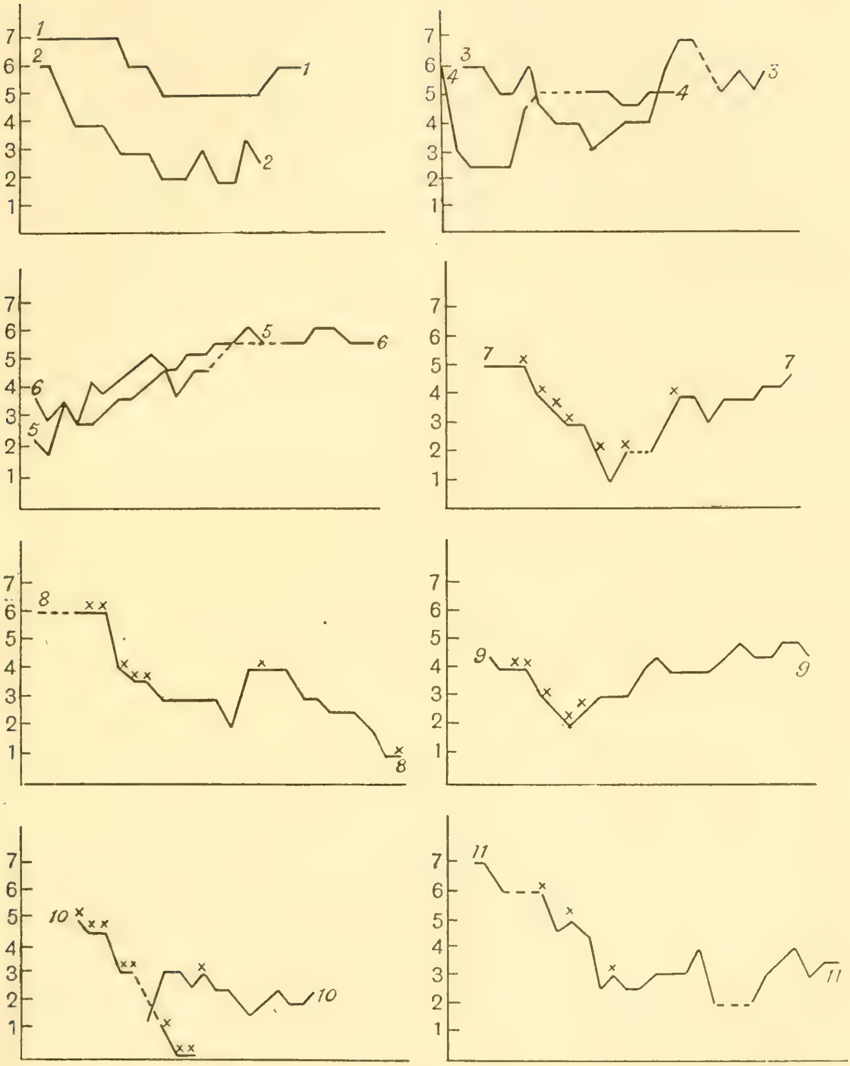


Fig. 18 Fig. 19 Fig. 20 Fig. 21 Fig. 18 *a* Fig. 19 *a* Fig. 20 *a* Fig. 21 *a* Fig. 22 Fig. 22 *a* Fig. 23 Fig. 23 *a*

Figs. 18–23*a*. Comparison of normal and reverted leaves. Figs. 18–22. First four leaves from apex of normal shoot. Figs. 18*a*–22*a*. First four leaves from apex of reverted shoot. Fig. 23. 10th leaf from apex of normal shoot. Fig. 23*a*. 10th leaf from apex of reverted shoot.

The following eleven cases have been selected from a number of similar ones from bushes growing at Long Ashton. The selection was made so as to reduce as far as possible the number of disturbing factors.



In each of the graphs 1–11, which correspond to the numbered cases considered in the text, the numbers in the ordinates indicate leaf vein numbers and the abscissae represent buds. Each graph starts from the basal leaf and proceeds upwards to the apex of the shoot. Where a leaf was missing the fact is indicated by a dotted line.

Table II.

1	2	3			4			5	6	
Veins	Veins	Veins	Mites	Leaf margin	Veins	Mites	Leaf margin	Veins	Veins	Mites
7	6	6	0	Normal	5	0	Normal	2 +	4	0
7	6	6	0	,,	3	3	Reverted	2	3	0
7	5	6	0	,,	2 +	0	,,	4	4	0
7	4	5	0	,,	2 +	0	,,	3	3	0
7	4	5	0	,,	2 +	0	,,	4 +	3	0
7	4	6	0	,,	2 +	0	,,	4	3 +	0
7	3	4 +	0	Reverting	4 +	0	,,	4 +	4	0
6	3	4	0	Reverted	Dam.	0	.	5	4	0
6	3	4	0	,,	5	0	,,	5 +	4 +	0
5	2	4	0	,,	Dam.	0	.	5	5	0
5	2	3	0	,,	5	0	Less rev.	5	4	0
5	2	3 +	0	,,	5	0	,,	5 +	5	0
5	3	4	0	,,	5	0	,,	5 +	5	0
5	2	4	0	Rather less	4 +	0	,,	6	Dam.	0
5	2	4	0	Hardly rev.	4 +	0	,,	6	6	0
5	4	6	0	Normal	5	0	,,	6 +	6	0
5	3	7	0	,,	5	0	,,	6	Missing	0
6	.	7	0	,,	5	0	,,	.	6	0
6	.	Missing	0	,,	.	0	.	.	6	0
6	.	5	0	,,	.	.	.	.	6	0
.	.	6	0	,,	.	.	.	.	6 +	0
.	.	5	0	,,	.	.	.	.	6 +	0
.	.	6	0	,,	.	.	.	.	6	.
.	.	.	0	.	.	.	.	.	6	.
.	.	.	.	.	.	.	.	.	6	.
.	.	.	.	.	.	.	.	.	.	0

7			8			9		
Veins	Mites	Leaf margin	Veins	Mites	Leaf margin	Veins	Mites	Leaf margin
Missing	0		6	10	Normal	Missing	Mod.	
,,	BB		Missing	4	,,	,,	,,	
5	BB	Normal	6	10	,,	4 +	,,	Nearly normal
5	BB	,,	6	6	,,	4	0	,,
5	BB	,,	6	15	,,	4	Mod.	Slightly rev.
5	0	Slightly rev.	6	0	,,	4	BB	,,
4	10	Reverted	4	0	Reverted	3	0	Very rev.
3 +	0	,,	3 +	0	,,	2 +	0	,,
3	BB	,,	3 +	0	,,	2	0	,,
3	0	,,	3	0	,,	2 +	0	,,
2	0	,,	3	0	,,	3	0	,,
1	0	Deformed	3	0	,,	3	0	,,
2	BB	Reverted	3	1	,,	3	0	,,
Missing	0	.	3	0	,,	4	0	Less rev.
2	0	,,	2	0	,,	4 +	0	,,
3	0	,,	4	0	,,	4	0	,,
4	0	Less rev.	4	0	,,	4	0	Normal in shape slightly reverted in margin
4	0	,,	4	0	,,	4	0	,,
3	0	More rev.	4	0	,,	4	0	,,
4	0	Less rev.	3	0	,,	4 +	0	,,
4	0	,,	3	0	,,	5	0	,,
4	0	,,	2 +	0	Oak leaf	4 +	0	,,
4 +	0	Semi-normal	2 +	1	,,	4 +	0	,,
4 +	0	,,	2 +	0	,,	5	0	,,
5	0	,,	2	0	,,	5	.	,,
.	0	.	1	0	,,	4 +	.	,,
.	.	.	1	0	,,	.	0	.
.	.	.	.	0	.	.	.	.

10			11		
Veins	Mites	Leaf margin	Veins	Mites	Leaf margin
Missing	Many		Missing	0	
,,	Mod.		7	0	Normal
,,	Many		7	BB	,,
5	,,	Slightly rev.	6	0	,,
4 +	,,	,,	Missing	Mod.	,,
4 +	,,	,,	,,	0	,,
3	,,	,,	6	0	,,
3	,,	Fully rev.	4 +	BB	,,
Missing		Second growth from here	5	0	Very rev.
1	,,	Oak leaf	4	0	,,
0	,,	,,	2 +	0	,,
0	Mod.	,,	3	0	,,
.	Many	.	2 +	0	,,
Second growth shoot			2 +	.	,,
Missing	0		3	.	,,
1	4	Reverted	3	.	,,
3	0	,,	3	.	Not rev. Irregular
3	0	,,	4	.	.
2 +	0	,,	2	.	Minute. First leaf of fresh growth
3	0	,,	Missing	0	.
2 +	0	,,	,,	0	.
2 +	0	,,	2	0	.
1 +	0	,,	3	0	Rev. margin but fairly broad
2	0	,,	3 +	0	,,
2 +	0	,,	4	0	,,
2	.	,,	3	0	,,
2	.	,,	3 +	.	,,
2 +	.	,,	3 +	.	.
.	0	.	.	.	.
.	.	.	.	0	.

They were all submitted to a more or less detailed analysis, notes being taken of the number of submain veins from the midrib running to points on the margin, of the mites present or absent in the corresponding axillary buds and of the character of the leaf margins (Table II).

Cases 1–6 were mite free, where examined for mite, with the exception of one bud in case 4, which for the moment may be regarded as negligible. Cases 7–11 were all affected with mite.

Case 1. This was a perfectly normal shoot. The seven first formed leaves, namely the basal ones, had seven submain veins, the next two six and then came a series of fives followed by three sixes. This shoot was examined on June 29th and had therefore by no means finished its growth. Graph 1 brings out the essential points more clearly. The drop from seven to five occurred largely during the month of June and possibly also during the end of May. This period, to judge by other growth graphs formed for apples and pears, constitutes the period of maximum growth. Now if, as previously indicated, a reduction of leaf veins occurs when the plant is subjected to a special growth stimulus, then a similar reduction should be expected, though less in degree, when the plant is under the normal growth stimulus which occurs under optimum conditions of growth. This is apparently what happens. At the same time the veins were never reduced below five, which may be taken as the lowest figure for normality, nor was the margin in the least reverted.

Case 2. While case 1 illustrates a leaf series of a normal shoot, case 2 represents the same for a reverted one. The twig indeed came off the same bush but from the reverted half. In this case the basal leaves were normal with six veins, but the successive leaves showed greater and greater reduction of veins and increase of reversion until the figure two was obtained for the tenth leaf (Table II and Graph 2). At the end of the graph there is a slight tendency for the vein number to recover, but by June 29th the growth season had not nearly finished. The graph shows therefore a much more marked descent than in Graph 1, but of the same order. Here therefore there appear to be two factors working, reversion and the normal drop due to growth stimulus.

Case 3. This was a shoot from a cutting from a seedling. All the rest of the bush was normal. No mite could be found in any of the lateral buds or in the terminal. Reference to Table II shows that the reversion effect came on fairly suddenly at leaf 7 where there was a sudden drop to 4 + in veins and the margin first showed distinct signs of reverting. This continued till leaf 14 when the margin began to show signs of improvement and by 16 was practically normal again. The vein number follows in the same line. Graph 3 shows much the same condition as Graph 2 except that being examined later, on July 7th, more of the terminal rising portion of the curve was obtained. Here therefore is a shoot that started normally and finished normally but in between was strongly reverted.

Case 4. This was a shoot from a bush that had been cut back for grafting and the shoot came from the stock. Again here the first leaf was normal, but the reversion was very sudden. Bud No. 2 contained three mites, but except for this the shoot was completely free from mite infection. The recovery in leaf vein number was fairly quick, the normal figure of five being attained by the ninth leaf after which there were only very small variations. The margin recovery was neither so quick nor so complete, there being indication of reversion here even in the last leaf formed.

Cases 5 *and* 6. Both these were very strong shoots, case 5 being a shoot from the base of a bush cut back for grafting and case 6 a shoot from a bush cut right down to the ground. Case 5 was not investigated for mite while none was found in case 6.

These two are considered together because their graphs (5 and 6) run practically together. Roughly speaking, only half the typical reversion curve, namely, the ascending half (of Graph 3), is represented. The reason that the first part of the curve is missed is probably this. Being hard cut back to weak buds growth starts relatively late in the spring and then is particularly vigorous owing to the upsetting of the balance between root and shoots. The late start also tends in the same direction, as general growth conditions are then fast approaching their maximum. However, the recovery of the venation is complete, in each case 6 or 6 + being attained. In one of these cases at least the issue was not directly affected by mite.

Case 7. In this and the following cases the position is complicated by the presence of mites in appreciable quantities.

In Table II the buds containing mites are indicated and an approximate figure given to indicate quantity.

In the graphs mite attack is indicated by a ×, but the position has been shifted in each case to four buds in advance of the one actually affected.

In Table II, No. 7 it is seen that the most basal bud was mite free, and that the next four buds were fairly heavily infected. The veins remained five and the margin normal until the sixth which shows a slightly reverted margin and the seventh which shows a definite drop in vein number below normality, a drop which was subsequently continued. If the mite infection therefore has anything to do with the production of reverted foliage no effect was produced until the fourth leaf subsequently produced. For this reason in the graphs of 7–11 the position of the mite infected bud has been marked four leaves forward. After these four big buds only single mite free buds were found for a space of four more buds and reversion gets steadily more marked up to the twelfth leaf which has a venation number of one and a deformed appearance. The next leaf which is four leaves from the last big bud has begun to recover and subsequent leaves up to 18 continue this gradual recovery, the vein number by this time being four and the margin distinctly "less reverted." The leaf following shows a sudden drop of vein number to three and the margin becomes distinctly "more reverted." The following or 20th leaf has again suddenly made a recovery to four and "less reverted" and this recovery is continued until the end leaf which has a vein number of five and a practically normal margin.

The behaviour of leaf 19 calls for an explanation. It stands as an island of greater reversion in a sea of lesser reversion in the same way that bud 13 stands as an island of mite infection in a sea of mite freedom. The hypothesis immediately suggests itself that the two facts are connected and that the isolated mite infection has caused an isolated drop back into the more reverted state. In this case the two spots are six buds away, not four. It is of course impossible to lay down any fixed number of buds which would separate a mited bud from its possible effect on the growing point. In practice this has been found to vary between three and six, and four has been selected as an average to apply to the graphs. Referring to Graph 7 the crosses (which indicate mite infection) are generally followed by an increase of reversion. At first sight one might argue that as a drop is always experienced in mite free reverted twigs at this period (*cf.* Graph 3) so the drop in Graph 7 was due to this cause alone. On the other hand the drop is far more marked than in mite free cases, the vein figure reaching one, while three is usually the lowest reached in a mite free specimen.

However this may be, the isolated mite infection occurring in bud 13 stands in a portion of the curve that should be ascending and nevertheless an increase of reversion follows shortly afterwards. It distinctly suggests a close connection between mite infection and reversion.

These changes are illustrated in Figs. 24–39. Fig. 24 is the third leaf from the base. It is quite normal in every way. Fig. 25 is the sixth leaf where reversion is just appearing in the margin though the venation number is still five. Figs. 26–31 illustrate leaves 7–12 and show the gradual reduction of the veins to one and the gradual coarsening of the lobes of the margin up to Fig. 30; the nettle-leaf appearance of the whole leaf has also been gradually increasing. Fig. 31 shows the most reverted leaf of the whole series, and as has already been shown in certain cases of temporary reversion such leaves tend to be deformed. It suggests a very strong interference with the normal physiology of the plant. From this point to the 17th leaf (Fig. 35) reversion became less. Figs. 31–35 represent this initial improvement (leaf 14 missing). In Fig. 36, representing leaf 19, the leaf vein number has dropped to three again and the whole margin appeared more reverted. The differences in the margin may be more clearly shown by analysing the figures for the leaf on each side. Thus leaves 17 and 20 (18 was practically identical with 17) have each seven points between the apex and the sinus, four of which receive submain veins, while leaf 19 (Fig. 36) has only six, of which three receive submain veins. There is therefore a distinct numerical

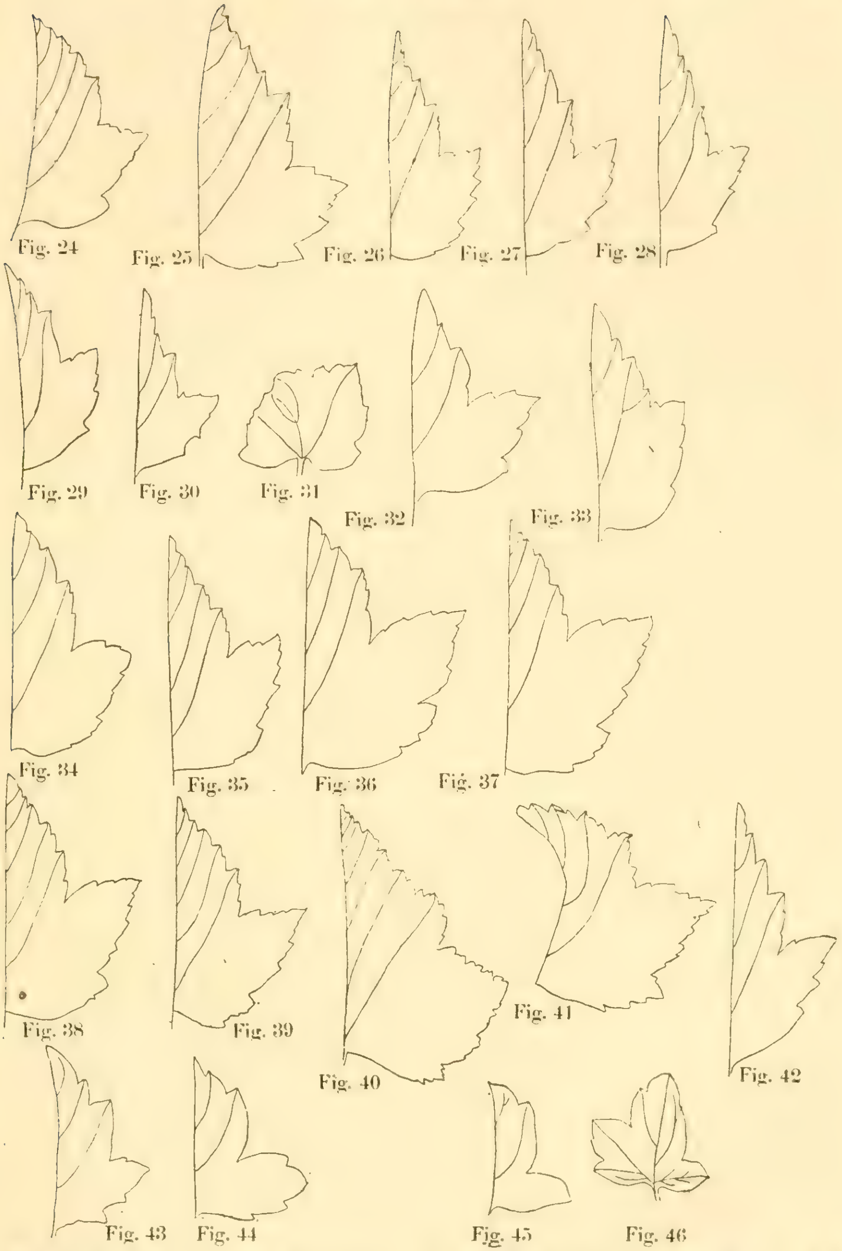


Fig. 24
Fig. 25
Fig. 26
Fig. 27
Fig. 28
Fig. 29
Fig. 30
Fig. 31
Fig. 32
Fig. 33
Fig. 34
Fig. 35
Fig. 36
Fig. 37
Fig. 38
Fig. 39
Fig. 40
Fig. 41
Fig. 42
Fig. 43
Fig. 44
Fig. 45
Fig. 46

Figs. 24–46. MITE INFECTED REVERSION. Figs. 24–39. Illustrate case 7. Fig. 24. 3rd leaf from base. Fig. 25. 6th leaf from base. Figs. 26–32. 7th–13th leaves from base. Figs. 33–35. 15th–17th leaves from base. Figs. 36–37. 19th–20th leaves from base. Fig. 38. 23rd leaf from base. Fig. 39. 25th leaf from base. Figs. 40–46. Series showing change from normal, through reverted, to oak leaf.

difference in addition to that readily perceived by the eye in the living specimen.

Unless the isolated mite infection in bud 13 be responsible for the sudden drop into the more reverted state there appears to be no reasonable explanation.

From this point onwards the recovery continues unchecked. The 20th, 23rd and 25th leaves are shown in Figs. 37–39, the latter being normal in vein number and nearly normal in margin.

Case 8. This was a reverted shoot from a half cut down bush examined on August 5th. The rest of the bush appeared normal. Here again, though the base of the shoot was mite infected, the first six leaves were in every way normal with a vein number of six. Reversion started quite suddenly at the 7th leaf on six buds in front of the first infected bud. The vein number suddenly dropped to four and the margin became reverted. Reversion gradually increased up to the 15th leaf with a vein number of two, after which a partial recovery to four set in for four buds, followed by further reversion to the end attended by the characteristic "oak leaf." As judged by the leaf vein number and margin, "oak leaves" appear to be merely an accentuated stage of reverted leaves. Such leaves (though not belonging to this series) are shown in Figs. 44–46.

The chief differences between case 8 and 7 are two in number. In Graph 7 the curve, with the exception of one bud, follows the general curve of reversion, the latter half having a general rising tendency. In Graph 8, though a short recovery begins at leaf 16, the general tendency of the latter part of the curve is downwards instead of upwards. The second point is that two isolated infected buds occurred after the basal infection. These were very slight as each only contained one mite.

Case 9. This was from an Ogden's Black bush with plenty of old big bud on the bush and was examined on August 6th.

This case shows in its graph the same sort of curve as does case 7, but there are no mite infected buds after the usual basal ones and the curve is of the same order as a reverted one unaffected by mites (of Graph 3). It is, however, different in that it drops lower, reaching a vein number of two, and also fails to reach so high a number at the end of the graph. Instead of attaining at this point a vein number of six or seven it scarcely reaches five and the leaf instead of being "normal" as in Graph 3 never gets beyond "normal in shape, but slightly reverted in margin." Though therefore of the same order as Graph 3 it is of different intensity and this difference may provisionally be referred to the mite infestation which is the only visible difference between the two shoots.

Case 10. This and the following one have been selected as special cases likely to throw light on the possible effect of mite infestation at the base. In both the shoot has ceased growth during the summer and a subsequent fresh growth has started from a bud later on. Case 10 was from a badly mite infested bush. It possessed five primary shoots of the current year and all contained mites in the terminal bud.

The particular shoot (10 in Table II) was examined on August 10th and bore twelve lateral buds, one of which, number nine, had subsequently grown out later in the season after the primary shoot had ceased growing. The graph for the primary shoot (Graph 10) showed a very quick descent to zero and reversion was so marked at the end that the leaves were strongly "oak leaf." If the hypothesis be accepted that mite infection produces reverted leaves, this was to be expected as the whole shoot was heavily infected. The secondary growth from bud 9 is shown in the lower half of number 10 in Table II. The first point to be noted is that bud 2 contained four mites. This was probably not a direct infection, but represented the residual mites in bud 9 of the primary shoot. It was probably due to the weakness of the infection that this bud grew out when growth conditions became urgent and not any other bud. The next point to be noted is that all the leaves are of the reverted type, though not so reverted as to become truly "oak leaf." The leaf vein number hovered about 2 + and showed no sign of a definite rise towards the end of the graph. The lower figure for bud 2 is probably due to temporary reversion due to growth conditions as indicated in the first part of this paper.

The conclusion that appears to be indicated is that this secondary shoot, though practically mite free and possessing none in the terminal bud, was under the influence of some special factor and that this factor was the high mite infestation of the primary shoot.

Case 11. This was from a young bush not heavily mite infected and examined August 31st. Its first seven leaves were normal with a high leaf vein number. Reversion began fairly suddenly at leaf 9 the margin showing up quite suddenly and the vein number quickly following in the next few leaves. Leaves 13 and 14 marked the bottom of the curve with a vein number of 2 +. From there, for four leaves the vein numbers and margin improved somewhat till at leaf 18 the shoot had ceased its first summer growth. As in the case of shoot 10, later on in the season a fresh growth began but this time it came from the terminal. This is of course the bud where one would expect a fresh growth, if anywhere, and no doubt it would have occurred from here in case 10 had it not been highly mite infected. In Table II, No. 11 a horizontal line is drawn at the spot where the first growth ceased.

Leaves 1 and 4 of the second growth shoot have the low vein number of two almost certainly owing to temporary reversion. Indeed, the whole shoot is not long enough to be certain that one has completely got rid of temporary reversion. On the whole the leaf vein numbers are on the low side though not so low as the secondary shoot of case 10. They indicate that they are still under the influence of the infected buds at the base of the primary shoot, but owing to the infestation being much less than in the case of the primary shoot of 10 the effect is also much less.

DISCUSSION.

These eleven cases may be divided into three classes, normal shoots as No. 1, reverted but mite free as Nos. 2–6, and reverted and mite infected as Nos. 7–11. With the exception of 5 and 6 all showed a vein number of about five (*i.e.* normality) at the beginning of growth. Both Nos. 5 and 6 were hard cut back shoots and these nearly always begin growth at a later period. Therefore it would appear that as a rule reverted bushes will commence the season with normal leaves.

Secondly all the shoots showed a drop in leaf vein number at the height of the growing season in May and June. The drop was slight in the normal shoots, well marked in non-infected reverts and still more marked in infected reverts. It would appear therefore that there are three factors at work, a seasonal one tending to reversion of a temporary kind, a reversion factor and a mite factor. One can of course never be sure unless one has carefully selected material that the fall in leaf vein number may not be due to the reversion factor only and not to the mite factor or that it may be due to both. In the mite infected cases above cited care was taken to select as far as possible only those shoots coming from bushes which otherwise appeared normal. Even supposing that all these cases would have proved reverted without mite infection, nevertheless the amount of fall is greater than in pure reversion cases and one may therefore presume that the mite infection has increased the effect. The same conclusion can be drawn from the behaviour of the ends of the graphs. Where the graph is long enough to show complete recovery the final leaf vein number is five or more for reverted cases (Nos. 3–6), but less where mite infection is not confined to the basal series (8). In 7 and 9 the recovery of the leaf vein number is practically complete, but the margin does not regain normality in either though it does in 3, the only reverted case with description of leaf margins completely free from mite. In 4, provisionally included in the non-mite infected, the recovery of margin was not complete even though the mite infection was very small. Similarly in 10–11, also mite infected cases, the leaf margin never completely recovered. Evidence pointing in the same direction may be found on consideration of the relation of "oak leaf" to mite infection. In all such cases so far examined mite has always been found though not by any means always in the terminal bud. It seems quite clear that the effect of a massive infection of mite is conveyed by some means to the terminal growing point when this is itself quite free from direct infection. The oak leaf effect has never been observed by the writer so far in bushes where no mite has been found, though reverted leaves of the usual type are common on mite free shoots in certain cases. Now before oak leaves are produced the shoot always makes ordinary reverted leaves as in the series of figures in Figs. 40–46. One may therefore hazard the opinion that the same cause which produces oak leaf will, when acting in greater dilution, simply produce reverted leaves. So conversely, when one finds reverted foliage unassociated with mite (other than temporary reversion) there is at least an indication that either the same cause, namely mite, had been at work and the mite has for some reason disappeared, or the plant is still suffering from a residuum of previous infection. If these speculations have any basis in fact, and the writer does not put them forth except as speculations, then it would appear that reversion is largely quantitative in action. That is to say, the effect will depend more on the dose than anything else. This seems to be borne out in the cases examined. Where, as in 7 and 8, isolated mite infected buds occur a depression shortly follows in the graph, even when this should be normally ascending. The secondary shoot in case 10 appears to be suffering in the same way from the load of mite in all the buds of the primary shoot.

The conclusions above arrived at are emphatically only preliminary. The evidence supporting them is largely circumstantial and the reasoning is largely in the backward direction. This was for the moment impossible to avoid as no selected material was at hand, and no material could be safely selected until an effective method of diagnosing slight cases of the disease had been found. This, the writer maintains, is furnished by the leaf vein and margin method. It is now possible to select absolutely healthy material during summer for experimental purposes for the following season. It is hoped therefore that it will be possible to do direct experimental work under controlled conditions and so avoid the pitfalls hitherto unavoidable.

ABSTRACT.

1. A means is indicated whereby reverted leaves may be identified even in very slight cases or where the leaves have almost regained normality.

This method depends (*a*) on counting the number of submain veins running from the midrib to points in the margin, and (*b*) on observation of the margin points, which also may if necessary be reduced to a numerical basis.

2. That reverted leaves may be produced by artificial means, but that this reversion is of a temporary character. In each case examined the plant appeared to be under a special stimulus to growth.

3. Cases in the field were examined in detail in three respects, namely (*a*) leaf vein number, (*b*) mite infected buds, and (*c*) leaf margin. Three classes could be distinguished: (*a*) normal healthy, (*b*) simple reverted, and (*c*) mite infected (reverted). These corresponded with three factors which appeared to be acting: (*a*) seasonal factor, (*b*) reversion factor, and (*c*) mite factor. Furthermore, since "oak leaf" is an advanced stage of reverted leaf and is always associated with mite, the chances are that reverted leaves when found without "oak leaf" owe their existence in some way or other to the mite factor either patently or latently.

EXPLANATION OF TABLES I AND II

In the columns headed "Veins" the numbers represent the number of submain veins from the midrib running to a point in the margin. The veins on only one side are counted, but where an extra one appears on either side the sign + is used after the numeral. Similarly the sign + is used where the topmost vein is doubtful. Thus 3+ indicates either 3 on one side and 4 on the other or that on one side there were 3 clearly defined veins and one doubtful.

The words "Missing" and "Dam." indicate that the leaf was absent or damaged.

In the column headed "Mites" the approximate number of mites in the particular axiliary bud is given. "Mod." signifies a moderate amount and "BB" that a big bud was already forming, this being an indication of heavy infestation. The last number refers to the terminal bud. In the column headed "Leaf margin" the character of the leaf margin is shown in accordance with the description in the text.

(*Received August* 31*st*, 1921.)

FURTHER OBSERVATIONS ON *SITONES LINEATUS* L.

By DOROTHY J. JACKSON, F.E.S.[1]

(With 2 Text-figures.)

In a previous article (1) a full description was given of the attack of *Sitones lineatus* L. upon peas and beans, and it was shown that these plants, together with tares and lucerne, constituted the favourite food plants of this species; clover being little attacked when they were available. In the end of July, 1921, large numbers of adults of this species were found feeding upon clover and lucerne in Kent, and the following observations on the damage thus effected may be of interest to record.

Owing to the long drought during the summer of 1921, the second growth of clover in the hay fields had made little progress and the leaves were seriously attacked by adults of *S. lineatus* L. The leaves growing on many of the flowering shoots were eaten nearly to the midribs (Fig. 1, *B*), and the younger foliage at the base of the plants had also suffered severely (Fig. 1, *C*). Lucerne was similarly damaged. The attack was always most severe in those fields of clover and lucerne which adjoined fields of peas or beans. The latter were by this time cut and mostly harvested.

The young clover coming up amongst the corn was also much attacked (Fig. 2) many of the leaves being completely eaten off, especially along the edges of the field next to fields of peas and beans. Where the corn had already been cut the clover had made less growth and appeared to have suffered more from the attack of the weevils.

The adults of *S. lineatus* L. were abundant around the damaged plants. They were to be found during the day running about amongst the withered leaves and stalks that littered the ground at the base of the plants. Only a few of the beetles occurred upon the foliage in the daytime. As soon as darkness set in they crawled up the stems and commenced feeding upon the leaves, and numbers were captured in the sweep net at this time.

All were freshly emerged specimens, sexually immature. Not a single individual of the old generation was observed. Without doubt the vast

---

[1] A grant in aid of publication has been received for this communication.

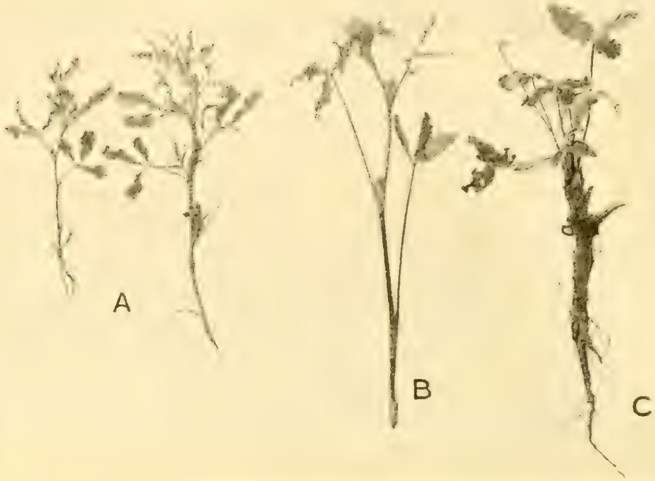


Fig. 1. Clover and lucerne with leaves partially destroyed by adults of *Sitones lineatus* L. *A* = lucerne (*Medicago sativa*), *B* = flowering shoot, and *C* = entire plant of red clover (*Trifolium pratense*) from aftermath of hayfield.

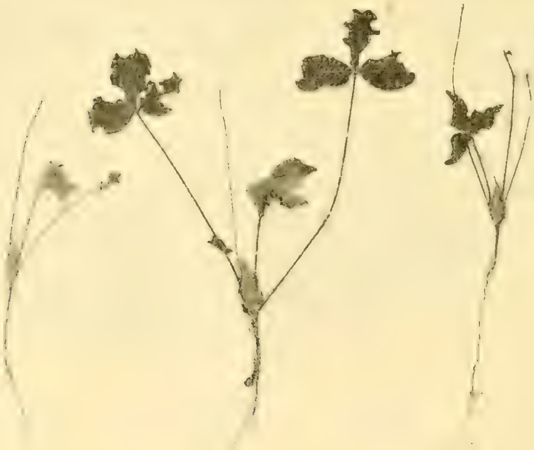


Fig. 2. Seedling plants of red clover with leaves destroyed by adults of *Sitones lineatus* L

majority of these beetles had been bred at the roots of peas, beans and tares and when these crops were cut had migrated to the clover and lucerne. It would appear unlikely that many of these beetles had been bred at the roots of the clover itself judging from the fact that the beetles are comparatively scarce upon clover in this district during the breeding season in spring, although they abound at this time upon peas, beans and tares, and also frequent lucerne. In the old pea and bean fields at the end of July many of the newly emerged beetles were still to be found amongst the stooks and when these were harvested a few remained under weeds upon the ground. They were also to be found amongst clover growing in permanent pastures and on waste ground. A certain number of other Sitones also occurred upon clover at this time. These included *S. puncticollis* Steph., *flavescens* Marsh, *sulcifrons* Thunb. and *hispidulus* F., species which (as will be shown in a subsequent article) live upon clover throughout the year and breed at its roots. Lucerne was also frequented by *S. hispidulus* and *S. crinitus* Herbst. These species contributed towards the general attack, but all were outnumbered by *S. lineatus* L.

REFERENCE.

(1) JACKSON, D. J. Bionomics of Weevils of the genus Sitones injurious to Leguminous Crops in Britain. *Ann. App. Biol.* VII, pp. 269–298.

(*Received September* 18*th*, 1921.)

CONTRIBUTIONS TO THE BIOLOGY OF FRESHWATER FISHES

By W. RUSHTON, A.R.C.S., D.I.C., F.L.S.

I. THE EFFECTS OF VARIOUS IMPURITIES IN A STREAM ON THE LIFE OF SPERMATOZOA OF TROUT AND YOUNG TROUT

At the suggestion of the owner of a salmon and trout stream in Banffshire, which of late years has become very polluted owing to various trade products, a series of experiments was undertaken to find out whether the various trade wastes have any effect on the fertilising power of the spermatozoa.

The method adopted was to have crude samples of every type of trade effluent collected when at its worst and delivered in London. On receipt of it the milt and ova were extracted from fully mature fish, and submitted to the various effluents in order to test the power of fertilisation in the presence of the impurity. When the milt and ova had been in contact a certain length of time the ova were placed in an artificial redd in running water and the number of eggs which hatched out and gave rise to normal embryos noted.

The whole of the work had to be done between December and March of the following year, as it is only in the late fall that fertile fish are obtainable. The trade effluents of the particular stream under consideration are waste products from six distilleries, consisting of spent malt products and yeast cells from the first distillations. Effluent from a tweed mill where wool-scouring, dyeing and weaving are carried on, and crude sewage from the town.

The effluent samples were collected as far as possible just as they were entering the stream except in the case of the cloth mills where this was impossible, owing to the mill standing over the stream, and only a diluted sample was obtainable.

The method of obtaining the ripe ova was to take mature brown trout and, after drying the fish as far as possible, by gentle pressure to extract the eggs into a clean dish; the same procedure was adopted in regard to the milt, great care being taken to prevent water mixing with either milt or eggs till the two were brought in contact in the presence of the effluent under examination.

Ten eggs were taken up in each case and submitted to the various effluents with a measured quantity of milt and the two left in contact for 30 minutes to ensure uniformity throughout.

Some of the milt was examined microscopically at the same time and the length of time before activity ceased was noted.

It is known that the milt of trout will remain in good condition and fertilise ova 24 hours after extraction if kept free from moisture, *i.e.* if care is taken in extraction that no drainings or drippings from the male fish are allowed to come in contact with it; but if brought in contact with water they at once become very active and lose their fertilising power after three minutes' activity so that the period of activity is very short. This experiment can easily be done by taking a series of drops of milt on a slide side by side and adding the smallest drop of water to each and the time of activity taken.

It is further known that some spermatozoa of fish are more sensitive to poisons than young or mature fish and may be killed by such small amounts of poisons as can be detected only by very refined chemical methods.

Sample 1. Water from the upper reaches of the stream before any effluent had entered.

Colour ... ... None.
Reaction ... ... Neutral cold or hot using phenol-phthalein indicator.
Activity of sperms Three minutes.

Eggs all fertilised and gave rise to normal embryos 90 per cent. of which hatched. One year and one two-year-old trout remained alive in this sample for over a week when they were removed to fresh water.

Sample 2. Containing effluent from one distillery.

Colour ... ... None.
Reaction ... ... Neutral hot or cold.
Sperms ... ... Active for 2 to 3 minutes.

Eggs all fertilised and gave rise to normal embryos 90 per cent. of which hatched out.

Sample 3. Diluted sample from a woollen mill where wool-scouring takes place.

Colour ... ... Slightly yellow.
Reaction ... ... Neutral cold, alkaline hot.
Sperms ... ... Active 2 minutes.

Ova fertilised and gave rise to normal embryos; 85 per cent. hatched out.

Sample 4. From same place as 3, but taken a few hours later; similar results to 3, but sample a little more alkaline. Two yearling trout introduced into these samples, although they showed uneasiness at first, soon settled down to the unusual conditions and were alive four weeks afterwards and during the period showed no unusual symptoms.

Sample 5. Taken from below the sewage outflow after crude sewage had entered the stream.

Colour	Yellow.
Reaction	Neutral cold, alkaline hot.
Sperms	Lived only 1·75 minutes.

Ova fertilised and gave rise to normal embryos in fresh water in four cases only. The effect on yearling fish was very marked with this sample. As soon as the fish were introduced they showed signs of trouble at once. The jaws began to move actively, attempts at leaping out of the liquid were very frequent, then after 5 minutes a period of rest at the bottom of the vessel, the fish appearing to be exhausted, then further attempts at coming to the surface were made with gradual sinking to the bottom again. This continued for 15 minutes, when the fish showed signs of turning on their sides and within a few seconds were on their backs. Then spasmodic darts took place in an aimless manner which continued periodically for about an hour when the fish died.

Repeating this experiment but removing to fresh water at the first signs of turning over it was found possible to recover them and in a few days they acted quite normal.

Diluting this sample 1 in 4 the period of being overcome was lengthened, but the final result was the same.

Sample 6. This was from the same source as 5, but taken from the outflow pipe before it entered the stream.

Analysis of this sample showed it to be crude sewage.

Colour	Brown.
Reaction	Distinctly alkaline cold or hot.
Smell	Very offensive, ammonia, sulphuretted hydrogen prevailing.
Analysis	Parts per 100,000.
Free and saline ammonia	5–6.
Albuminoid ammonia	1–1·1.
Oxygen absorbed in two hours at 27° C.	8–9.

Sample shaken up showed froth at top which remained for 24 hours before disappearing (in a purified sample froth should disappear in three minutes). Sperms were active in this sample for one minute only. Ova fertilised in this crude sewage and removed to fresh water gave normal embryos, only four hatching out.

Yearlings placed in this sample would not live more than 5 minutes and throughout the period of emersion were in a violent state of activity.

Sample 7. This was a sample taken from the effluent pipe from a distillery when a full discharge was taking place.

A large amount of suspended colloidal matter was present which microscopic examination showed to be broken-down yeast cells and starch grains, some in an unbroken condition and others much broken up. The whole was of a whitish colour with a large amount of finely suspended matter very like starch paste.

The cloudiness took several days to disappear on standing when a mass of fungal hyphae appeared on the bottom.

Reaction ... ... Slightly acid cold; neutral hot.

Tested for presence of CO_2 showed a large amount present. Milt and ova were not available when this sample was taken and only its effects on yearling trout was ascertained.

This was peculiar, for almost as soon as introduced the fish became very sleepy, remaining at the bottom of the vessel till finally overcome within an hour and if left half an hour longer died.

Repeating this experiment but removing to fresh water at the end of an hour, when first overcome, the fish recovered its normal position within 10 minutes, indicating that crude distillery waste puts fish out of action probably through the excess of CO_2 present and the deficiency of free oxygen. This experiment has been repeated using freshly drawn distillery wash from a London distillery and the same effect obtained.

The amount of oxygen in solution rarely reached more than 2 c.c. per litre against a normal 6–7. Well aerating a sample of distillery wash improved matters especially after standing for some time to allow the colloidal matter to settle out. The addition of lime water or powdered lime helped to improve the liquor so that it was able to support fish-life if most of the CO_2 was removed and oxygen introduced.

SUMMARY OF RESULTS.

Sample	Colour	Reaction	Debris	Life of sperms mins.	Effect on fish	Remarks
1. Pure water	None	Neutral	None	3	Unaffected	90 % eggs hatched out
2. Diluted distillery effluent	None	Hot or cold neutral	None	2–3	,,	1 distillery, 90 % eggs hatched out
3. Diluted woollen mill effluent	Slightly yellow	Neutral cold, alkaline hot	Small amount	2·0	Slightly affected	85 % hatched out
4. Diluted woollen mill effluent	Slightly yellow	Alkaline, hot or cold	Small amount	2·0	Uneasiness	85 % hatched out
5. Sewage	Yellow	Neutral cold, alkaline hot	Small amount	1·75	Fatal	Contained large percentage sewage
6. Crude sewage	Brown	Alkaline cold, alkaline hot	Small amount	1·0	Fatal	Very offensive, putrid smell, 40 % eggs hatched out
7. Crude distillery waste	Whitish	Slightly acid cold, neutral hot	Small amount	—	Fatal	Contained a lot of col loidal matter, burs yeast and starch grai

Field-work was done in the area from which the samples were drawn to determine how far the laboratory experiments agreed with the conditions present.

The distillery effluent has a very marked effect on the bed of the stream.

A very small tributary of the main stream on the banks of which only one distillery is present was selected.

Above the distillery the water was clear and fish were abundant, below the distillery for about a mile no fish were found. The distillery effluent is allowed to settle a little but reaches the stream as a yeast-coloured liquid. The stream is coloured a short distance below outflow and as the debris settles a filamentous fungus appears covering the stones with a grey flocculent growth.

The species of fungus was not determined but it covers the stones for over a mile down the stream when it gradually thins out where the stream becomes normal again and fish appear. When the fungus has fructified it turns black and gives rise to a black slimy mud of a very offensive nature and makes the stream look black.

The distillery effluent contains a large amount of nitrogen from the yeast and barley and unused up starch grains. Below the woollen mill no fish were present on account of the high alkalinity which prevails at times together with waste dye-stuffs. The crude sewage gives rise to fungal growths very similar to that from the distilleries, but in less amounts.

CONCLUSIONS.

1. That distillery effluent and crude sewage is detrimental to the life of sperms and fish if poured into a stream untreated.

2. That the contents of distillery effluent give rise to fungal growths, preventing algal and flowering plants from growing and aerating the water.

3. That the plant and animal life of a stream is affected by crude trade wastes and untreated sewage entering it.

II. BIOLOGICAL PROBLEMS CONNECTED WITH A TROUT FARM

Some two years ago my attention was drawn to a serious trouble, which frequently occurs in the rearing of trout for re-stocking streams, at a trout farm in the south of Scotland; and as a result of the investigation appeared to be of economic importance, it was thought worth while to record it.

The disease is one only found in northern areas and known as "Bloom." It attacks young fry a few weeks after hatching when the food-sac is all used up and artificial feeding has begun. Often the disease continues all through the summer, only fry which are mildly attacked surviving.

The attack takes the form of a bluish appearance, arising on the flanks of the fish just behind the gill covers, gradually extending backwards towards the tail, during which time the fish get perceptably thinner and ultimately succumb a few weeks after the first attack.

The hatchery in question is served by two streams from which raceways direct the water to the various parts of the farm. Both streams drain uncultivated hillsides, and the water in both cases is the usual brown colour common to Scottish burns. After passing through the hatchery, the water returns to the main stream and flows away to the sea.

The subsoil from which the streams draw their water is of granite covered with a thick layer of peat, and the volume of water flowing through the hatchery is about 300,000 gallons per hour.

The hatchery is situated in a hollow completely surrounded with hills one of which slopes down into the grounds, and in pre-war days was covered with spruce which has since been removed. The conditions of the water draining from this area being considerably altered in consequence.

At the base of this hill a large number of sphagnum bogs occur and the water passing through these bogs, though perfectly clear, has a very detrimental effect on fish-life as repeated experiments have shown.

It does not seem to make much difference whether the experiment is tried before or after a period of drought, or in spring or summer, the result is the same, and yet not a yard separates the burn from the bog-water at some places.

Before deforestation of the hillside, the surface water was allowed to mix with that of the burn water running into the hatchery, but on account of its deadly character an extensive process of draining has been undertaken to prevent any of it reaching the water of the hatchery.

It may be a coincidence, but the altered character of the surface drainings since deforestation is very marked.

In a later paper I hope to deal more fully with this sphagnum water.

BLOOM.

The symptoms of this disease have already been mentioned. It can easily be removed by putting the fry into a solution of common salt about 5 per cent. strength, but the bloom soon returns if the fish are put back into the same water from which they were drawn.

Microscopic examination of the bloom gives no clue as to its composition as it appears as a homogeneous mass of slime, no bacteria are present, nor do cultures from this slime give any positive results. Chemical investigations show it to be coagulated mucous due to the high acidity of the peat water at times, and the presence of vegetable toxins.

It has been stated that the hatchery is served by two streams, one draining a small area and the other a larger one. Taking the acidity of the two streams after a period of normal steady weather, and using phenolphthalein as indicator and boiling the samples before titrating, the acidity of the two streams calculated as sulphuric acid give:

Stream from large area, 3·62 parts acid per 100,000
Stream from small area, 1·03 ,, ,,

It was found necessary to use N 100, NaOH and 200 c.c. quantities of water to get a good end point of the acidity.

It is found that the acidity varies considerably over long periods; given a period of settled weather the acidity rarely varies from the above, but after a storm (say two or three hours) the acidity drops quickly for about an hour, then rises quickly; within an hour I have known it rise to three or four times above the normal. The temperature may fall a degree or two but not to any marked extent.

After the acidity has risen it remains high for some hours and then very gradually comes down, taking often several days to get anywhere near the normal figure.

It is known by long experience that sudden changes in the weather are very trying for fish under artificial conditions and many young fish are lost on this account, older fish being able to withstand the changes much better than the young ones.

It has been found that the bloom makes its appearance immediately after this sudden rise in acidity, and from all experiments tried on the spot (and repeating as near as possible in glass tanks the condition of sudden rise in acidity, keeping the fish under observation all the time) it is found possible to produce artificially a somewhat similar appearance to what occurs at this hatchery, not forgetting that the water at the hatchery also contains a certain amount of vegetable matter in suspension and a certain number of vegetable toxins.

Dachnowsky in the *Botanical Gazette*, 1909, gives an account of vegetable toxins which are detrimental to animal and plant life and which occur in peat water at different times of the year.

Examination of a large number of fish which have died through bloom always shows the mouth and gill covers extended to their widest extent with the tips of the gill filaments covered with a thick layer of coagulated mucous, the stomach is invariably swollen, containing no food but varying amounts of coagulated mucous.

The first quarter of an inch of the intestine immediately following the pyloric end of the stomach is usually inflammed, due, I take it, to the high acidity of the contents of the stomach set up by the acid mucous.

Many experiments have been tried to effect a cure both on the spot and in the laboratory. Various reagents have been tried, but the most effective appears to be powdered lime, chalk, or lime water.

It has been found that causing the water to run through chalk filters, which hardens the water a little and brings down the acidity, is effectual; as is also the careful addition of lime to the water at a point where no free lime would be able to reach the hatchery. But the easiest and the most economical way has been by using lime water.

Calculating the average rate of flow and the highest acidity known to occur, it has been found possible by turning some of the ponds into lime-pits, and arranging that a certain amount of lime water flows in gradually to so regulate matters that this bloom no longer appears.

The questions of the variation and composition of the dissolved gases are now under consideration, together with the composition of the water at varying seasons.

The conclusions to be drawn from this series of observations are:

1. That deforestation considerably alters the character of the water flowing from hillsides so far as fish life is concerned.
2. That the altered water in this instance has a very detrimental effect on fish life especially in the young stages.
3. That too high acidity of the peat water may cause the coagulation of the mucous on the gills, and sides of the fish, which may be fatal.
4. That a careful control and adjustment of the acidity of the water is necessary to ensure the non-appearance of this bloom, and lime water or chalk is the most effective.

(*Received January* 19*th*, 1922.)

OBITUARY NOTICE

Dr CAROLINE BURLING THOMPSON.

1869–1921.

Dr Caroline Burling Thompson, Professor of Zoology at Wellesley College, Mass., U.S.A., died on December 5, 1921. Prof. Thompson was noted not only for the excellence and thoroughness of her original methods of teaching, but also for her original research work in biology. She was an inspiration to her students and also found means of helping them in many practical ways, unknown to any but herself.

Miss Thompson did original research work in biology in connection with the marine laboratories both at Naples, Italy, and Woods Hole, Mass. Her most noted work was on the biology of termites—the most destructive of the social insects. She has been a Collaborator of the Branch of Forest Entomology, Bureau of Entomology, U.S. Department of Agriculture, since March 1917.

1916 saw Miss Thompson's first paper on termites. It was an original piece of research on the brain and frontal gland of a common termite of eastern United States. She discovered that there was very little differentiation between the brains of the different castes of this termite and none between the sexes, the most marked difference being in the optic apparatus. Miss Thompson suggests that the frontal gland may have arisen phylogenetically from the ancestral median ocellus now lacking. This work was of considerable importance, since the frontal gland is of great taxonomic value.

In 1917, a paper on the origin of the castes of a common termite revolutionised the attitude taken by students of termites. Hitherto the attitude had been almost entirely anthropocentric; Dr Thompson disproved that the "complementary" or "substitute" queens or reproductive forms of termites could be manufactured through feeding by workers. She definitely proved that the origin of all castes is due to intrinsic causes. Thus, by careful scientific study, much of the mystery of the "complex" social system of the termites—which has led to admiration by man of these insects—has been proven a myth. Facts now supplant the older fantastic theories, so dear to writers of the eighteenth and nineteenth centuries!

Another paper in 1919 discussed the phylogeny of the termite castes and outlined breeding experiments which were in progress at the time of her death. It was hoped to work out a genetic formula for termites.

These papers were followed by several others on the development of the castes and reproductive forms of species of many genera of termites.

Work on the development of the castes of the honey bee had been planned and material fixed ready to section. It is to be regretted that ill-health and other duties interfered. Miss Thompson was undertaking this work as she ever did with an open mind—realising that very careful work had been done on the honey bee and that no generalisations could be made in advance. The social insects often radically differ in habits. What might be an anthropocentrism in case of the termites, might be a fact in the biology of the honey bee!

With two other co-workers, Miss Thompson was working on a more or less popular book on termites and her share was to be the internal anatomy of termites as well as phylogeny and genetic work.

A kindly, helpful spirit, of keen mind, but modest—Miss Thompson will be long remembered by her students and co-workers in science. A striking point in Dr Thompson's personality, in fact its keynote, and which signalised her as an investigator and as a teacher, is that with all her splendid training and her admirable technique she was not biased by the current fashions of the school in which she was trained but struck out into new fields. Her own research work will endure for ever!

F. E. SNYDER,

Specialist in Forest Entomology, United States
Department of Agriculture.

LIST OF MEMBERS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR 1921–2

Honorary Members

BERLESE, Prof. Dr ANTONIO, *R. Staz. di Entomologia Agraria, Firenze, Italy.*
BOS, Prof. Dr J. RITZEMA, *Willie Commelin Scholten, Amsterdam, Netherlands.*
HOPKINS, A. D., PH.D., *Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.*
HOWARD, Dr L. O., *Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.*
JOHANNSEN, Prof. W., *Plant Physiology Laboratories, The University of Copenhagen, Denmark.*
MARCHAL, Prof. P., *Institut National Agronomique,* 16, *Rue Claude Bernard, Paris.*
RAILLIET, Prof., *Alfort, Paris.*

Ordinary Members

1914 ADAIR, E. W., *Department of Agriculture, Cairo.*
1920 ADAMS, Miss E. F. M., B.SC., *Welsh Plant Breeding Station, Agricultura Buildings, University of Wales, Aberystwyth.*
1921 ADAMSON, R. S., M.A., B.SC., *Botany School, The University, Manchester.*
1920 ALCOCK, Mrs A. L., *Ministry of Agriculture, Milton Road, Harpenden, Herts.*
1914 ANSTEAD, R. D., *United Planters' Association, Bangalore, Southern India.*
1908 ASHWORTH, Prof. J. H., D.SC., F.Z.S., F.R.S., 69, *Braid Avenue, Edinburgh.*
1914 AWATI, P. R., *Central Research Institute, Kasauli, India.*
1920 BACOT, A. W., *Lister Institute, Chelsea Gardens, London, S.W.* 1.
1914 BAILEY, M. A., 49, *Alleyn Park, Dulwich.*
1919 BALLS, W. LAWRENCE, SC.D., M.A., F.L.S., *Fine Cotton Spinners' Association, Rock Bank, Bollington, Nr. Macclesfield, Cheshire.*
1914 BARKER, Prof. B. T. P., M.A., *National Fruit and Cider Institute, Long Ashton, Bristol.*
1920 BAYLISS ELLIOTT, Mrs J. S., D.SC., *Botany School, The University, Birmingham.*
1922 BEDFORD, H. W., *W.I.R. Laboratories, Khartoum, Sudan.*
1919 BEER, R., B.SC., F.L.S., *Westwood, Bickley, Kent.*
1920 BENSON, Prof. MARGARET, D.SC., F.L.S., *Botany School, Royal Holloway College, Englefield Green, Surrey.*
1920 BERRIDGE, Miss E. M., D.SC., F.L.S., *Botany Department, Imperial College of Science, London, S.W.* 7.
1919 BEWLEY, W. F., D.SC., *Research and Experimental Station, Cheshunt, Herts.*
1915 BIJL, Prof. P. A. VAN DER, M.A., D.SC., F.L.S., *The University, Stellenbosch, Union of S. Africa.*
1914 BILLINGHURST, H. G., F.R.M.S., 76, *Lebanon Gardens, Wandsworth, S.W.* 18.
1919 BINTNER, J., *Helmdange, Grand Duché de Luxembourg.*
1920 BLACKMAN, F. F., M.A., SC.D., F.R.S., *St John's College, Cambridge.*

1919 Blackman, Prof. V. H., M.A., Sc.D., F.R.S., *Imperial College of Science, S.W.* 7.
1921 Bledisloe of Lydney, The Right Hon. Charles Lord, K.B.E., M.A., M.R.A.C., F.C.S., F.R.Stat.Soc., *Lydney Park, Gloucestershire.*
1909 Bles, E. J., D.Sc., *Elterholm, Madingley Road, Cambridge.*
1916 Bocock, C. H., F.E.S., *The Elms, Ashley, Newmarket.*
1920 Borthwick, A. W., O.B.E., D.Sc., *Forestry Commission,* 25, *Drumsheugh Gardens, Edinburgh.*
1920 Boycott, Prof. A. E., M.A., D.M., F.R.S., 17, *Loom Lane, Radlett, Herts.*
1920 Brade-Birks, Rev. S. Graham, M.Sc., *S.E. Agricultural College, Wye, Ashford, Kent.*
1920 Breeze, Miss B. M., 1, *Victoria Street, Emmanuel Street, Cambridge.*
1919 Brenchley, Miss W. E., D.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*
1914 Brierley, William B., D.Sc., F.L.S., F.R.A.I., *Rothamsted Experimental Station, Harpenden, Herts.*
1920 Bristol, Miss B. M., D.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*
1914 Brooks, F. T., M.A., *The Botany School, Cambridge.*
1921 Brooks, R. St John, M.D., M.A., D.P.H., D.T.M. & H., *Lister Institute, Chelsea Gardens, London, S.W.* 1.
1920 Bruce, A. B., M.A., *Ministry of Agriculture,* 10, *Whitehall Place, London, S.W.* 1.
1920 Buddin, W., M.A., *Laboratory of Plant Pathology, University College, Reading.*
1914 Burns, W., *Office of Economic Botanist, Agricultural College, Poona, India.*
1920 Butler, E. J., D.Sc., C.I.E., M.B., F.L.S., *Imperial Bureau of Mycology,* 17, *The Green, Kew, Surrey.*
1920 Campbell, A. V., *Star House, Ripley, Harrogate.*
Orig. Carpenter, Prof. G. H., D.Sc., *Royal College of Science, Dublin, Ireland.*
1914 Cayley, Miss D. M., *John Innes Horticultural Institute, Merton, Surrey, S.W.* 19.
1905 Chandler, S. E., D.Sc., F.L.S., *Imperial Institute, London, S.W.* 7.
1919 Chipp, Major T. F., B.Sc., *Forestry Department, Coomassie, Gold Coast.*
1908 Chittenden, F. J., F.L.S., V.M.H., *The Laboratory, R.H.S. Gardens, Wisley, Ripley, Surrey.*
1921 Chrystal, R. N., B.Sc., *Forestry Commission,* 22, *Grosvenor Gardens, London, S.W.* 1.
1905 Cornwallis, F. S. W., *Linton Park, Maidstone, Kent.*
1915 Cotton, A. D., F.L.S., *Herbarium, Royal Botanic Gardens, Kew, Surrey.*
1918 Cragg, P. A., *Merivale Nurseries, Heston, Middlesex.*
1920 Cunliffe, N., *Research Institute, School of Forestry, Oxford.*
1920 Cutler, D. W., M.A., F.L.S., *Rothamsted Experimental Station, Harpenden, Herts.*
1920 Cutting, E. M., M.A., F.L.S., *Botany School, University College, Gower Street, London, W.C.* 1.
1920 Darbishire, Prof. O. V., Ph.D., B.A., F.L.S., *Botany School, The University, Bristol.*
1920 Davey, Miss A. J., M.Sc., *Botany School, University College, Bangor.*
1915 Davidson, J., D.Sc., F.L.S., F.E.S., *Rothamsted Experimental Station, Harpenden, Herts.*
1920 Deacock, R. J., B.Sc., *School House, Crundale, Canterbury.*
1914 Deakin, R. H., *Joan Cottage, Bamford, Derbyshire.*
1920 Delf, Miss E. M., B.A., D.Sc., F.L.S., *Westfield College, Hampstead, N.W.* 3.

1915 Doidge, Miss E. M., M.A., D.Sc., F.L.S., *Division of Botany, Department of Agriculture, Pretoria, South Africa.*
1920 Dowson, W. J., M.A., F.L.S., *R.H.S. Gardens, Wisley, Ripley, Surrey.*
1920 Drummond, J. M., M.A., F.L.S., *Scottish Society for Research in Plant Breeding, Craigs House, Corstophine, Midlothian.*
1920 Duffield, C. A. W., F.E.S., *Stowting Rectory, Nr. Hythe, Kent.*
1920 Dykes, Wm. R., M.A., L.-ès.-L., *Royal Horticultural Society, Vincent Square, London, S.W.* 1.
1917 Eltringham, H., M.A., D.Sc., F.E.S., 6, *Museum Road, Oxford.*
1914 Emptage, W. F., 1, *Ullswater Road, West Norwood, S.E.* 27.
1916 Eyre, J. A. Vargas, D.Sc., *The Linen Industry Research Assoc., The Research Institute, Lambeg, Belfast.*
1920 Fahmy, T., 63, *Avenue Shoubra, Cairo.*
1909 Farmer, Prof. J. B., M.A., D.Sc., F.R.S., *Imperial College of Science, London, S.W.* 7.
1920 Fenton, E. Wyllie, *Botanical Department, Seale-Hayne Agricultural College, Newton Abbott, Devon.*
1920 Fisher, R. A., M.A., *Rothamsted Experimental Station, Harpenden, Herts.*
1905 Freeman, W. G., A.R.C.S., B.Sc., *Royal Botanic Gardens, Trinidad.*
1919 Fryer, C. H., 11, *St Mary's Road, Tonbridge, Kent.*
1913 Fryer, J. C. F., M.A., F.E.S., *Pathological Laboratory, Milton Road, Harpenden, Herts.*
1919 Fryer, P. J., F.I.C., *Ravenscar, Tonbridge, Kent.*
1918 Gahan, C. J., M.A., D.Sc., F.E.S., *Natural History Museum, London, S.W.* 7.
1914 Gamble, Prof. F. W., D.Sc., F.R.S., *The University, Edmund Street, Birmingham.*
1914 Gardiner, Prof. J. S., M.A., F.R.S., *Bredon House, Selwyn Gardens, Cambridge.*
1920 Gates, Prof. R. R., B.Sc., Ph.D., F.L.S., *King's College, Strand, London, W.C.* 2.
1922 Gilchrist, Miss G. G., B.Sc., *Botanical Department, The University, Bristol.*
1920 Gimingham, C. T., O.B.E., F.I.C., F.E.S., *The Bury, Offchurch, Leamington.*
1920 Glynne, Miss M. D., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*
1920 Goodey, T., D.Sc., *School of Tropical Medicine, Endsleigh Gardens, Euston Road, London, W.C.* 1.
1908 Gough, G. C., B.Sc., 45, *Poplar Avenue, Edgbaston, Birmingham.*
1914 Gough, L. H., Ph.D., F.E.S., *Agricultural Department, Cairo, Egypt.*
1921 Gray, P. H. H., M.A., *Rothamsted Experimental Station, Harpenden, Herts.*
1905 Green, E. E., F.E.S., *Way's End, Camberley, Surrey.*
1909 Groom, Prof. P., M.A., D.Sc., *Imperial College of Science, South Kensington, S.W.* 7.
1921 Grubb, N. H., M.Sc., *East Malling Research Station, East Malling, Kent.*
1921 Guillibaud, W. H., B.A., *Forestry Commission*, 22, *Grosvenor Gardens, London, S.W.* 1.
1914 Güssow, H. T., F.R.M.S., 43, *Fairmount Avenue, Ottawa, Ontario, Canada.*
1920 Gwynne-Vaughan, Prof. Dame Helen, D.B.E., D.Sc., LL.D., F.L.S., *Botanical Department, Birkbeck College, Chancery Lane, E.C.*
1909 Hadwen, Seymour, D.V.Sci., *Dominion Experimental Farm, Agassiz, Canada.*
1920 Halket, Miss A. C., B.Sc., *Bedford College, Regent's Park, London, N.W.*
1914 Hamilton, Dr Lillias, *Agricultural College for Women, Studley.*

1920 Hampshire, P., F.R.M.S., *Bureau of Bio-Technology, 3 and 4, Queen's Square, Leeds.*

1921 Harland, S. C., D.Sc., *Shirley Institute, Didsbury, Manchester.*

1920 Henderson-Smith, J., M.B., Ch.B., B.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Hiley, W. E., M.A., *Research Institute, School of Forestry, Oxford.*

1920 Hill, A. W., Sc.D., M.A., F.R.S., *Royal Botanic Gardens, Kew, Surrey.*

1920 Hiscox, Miss E. R., B.Sc., *Research Institute in Dairying, University College, Reading.*

1920 Holden, H. S., D.Sc., F.L.S., *University College, Nottingham.*

1920 Hole, R. S., M.A., C.I.E., *c/o Messrs H. S. King and Co.*, 9, *Pall Mall, London, S.W.* 1.

1919 Horne, A. S., D.Sc., F.L.S., F.G.S., *Botanical Department, Imperial College of Science, South Kensington, S.W.* 7.

1920 Horton, E., B.Sc., F.I.C., *S.E. Agricultural College, Wye, Kent.*

1920 Hunter, B. K., *Cauldron Barn Farm, Swanage, Dorset.*

1914 Hutchinson, H. P., *The Laurels, Kegworth, Derby.*

Orig. Imms, A. D., M.A., D.Sc., F.L.S., F.E.S., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Isaac, P. V., B.A., F.E.S., 2, *Gledhow Terrace, South Kensington, London, S.W.* 7.

1918 Jackson, Miss D. J., *Swordale, Evanton, Ross-shire.*

1907 Jepson, F. P., M.A., F.E.S., *Department of Agriculture, Peradeniya, Ceylon.*

1920 Jewson, Miss S. T., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Jones, Prof. W. Neilson, M.A., F.L.S., *Bedford College, Regent's Park, London, N.W.*

1918 Kannan, Kunui, M.A., F.E.S., *Asst. Entomologist, Govt. of Mysore, Bangalore, S. India.*

1915 Keeble, Prof. F., C.B.E., M.A., Sc.D., F.R.S., *Botany School, Oxford.*

1920 Kidd, F., M.A., D.Sc., *Botany School, Cambridge.*

1907 King, H. H., F.L.S., F.E.S., *Gordon College, Khartoum, Egypt.*

1907 King, Prof. L. A. L., M.A., *West of Scotland Agricultural College, Blythswood Square, Glasgow.*

1921 Lacey, Miss M. S., B.Sc., *Botanical Department, Imperial College of Science, London, S.W.* 7.

1921 Lamb, S. C., *c/o Chivers and Sons, Ltd., Histon, Cambridge.*

1921 Laurie, Prof. R. D., M.A., F.Z.S., *University College of North Wales, Aberystwyth.*

1908 Lees, A. H., M.A., *National Fruit and Cider Institute, Long Ashton, Bristol.*

1909 Leiper, Prof. R. T., M.D., D.Sc., *School of Tropical Medicine, Endsleigh Gardens, Euston Road, London, W.C.* 1.

1914 Lesley, J. W., M.A., *Emmanuel College, Cambridge.*

1915 Lister, A. B., B.Sc., D.I.C., *Experiment Station, Turner's Hill, Cheshunt, Herts.*

1921 Lobjoit, William, *Ministry of Agriculture*, 4, *Whitehall Place, London.*

1922 Lowe, L. T., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1919 Lloyd, Llewellyn, D.Sc., *Cartref, Slingsby, Malton, Yorks.*

Orig. MacDougall, Prof. R. S., M.A., D.Sc., F.E.S., F.R.S.E., 9, *Dryden Place, Edinburgh.*

1914 McClellan, F. C., M.R.A.C., F.L.S., *Director of Agriculture, Zanzibar.*

1920 McKenzie, Miss A. D., *Ministry of Agriculture*, 10, *Whitehall Place, London, S.W.* 1.

1920 McLean, Prof. R. C., M.A., D.Sc., F.L.S., *Botany School, University College, Cardiff.*

1920 McLean Thompson, Prof. J., M.A., D.Sc., F.R.S.E., *Botany School, The University, Liverpool.*

1909 Mangan, Prof. J., M.A., *University College, Galway.*

1919 Mangham, Prof. S., M.A., *Botany School, University College, Southampton.*

1917 Mann, H. H., D.Sc., F.L.S., *Agricultural College, Poona, India.*

1914 Marshall, G. A. K., C.M.G., D.Sc., F.Z.S., F.E.S., *Imperial Bureau of Entomology, Natural History Museum, London, S.W.* 7.

1920 Mason, Francis Archibald, 29, *Frankland Terrace, Leopold Street, Leeds.*

1921 Mason, T. G., M.A., B.Sc., *Imperial Department of Agriculture, Barbadoes.*

1920 Mattick, A. T. R., B.Sc., *Research Institute in Dairying, University College, Reading.*

1920 Mellor, J. E. M., B.A., 51, *Onslow Square, London, S.W.* 7.

1921 Millard, W. A., B.Sc., *Department of Agriculture, The University, Leeds.*

1914 Moore, Sir F. W., M.R.I.A., V.M.H., *Royal Botanical Gardens, Glasnevin, Dublin.*

1922 Morland, M. T., M.A., *Pathological Laboratory, Milton Road, Harpenden.*

1920 Morris, H. M., M.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Mosley, F. O., F.L.S., *Laboratory of Plant Pathology, University College, Reading.*

1914 Munro, Dr J. W., *Forestry Commission, Royal Botanic Gardens, Kew, Surrey.*

1920 Murphy, A. J., 2, *Dorset Square, London, N.W.* 1.

1920 Murphy, P. A., B.A., *Seeds and Plant Disease Division, Royal College of Science, Dublin, Ireland.*

1914 Neave, S. A., M.A., D.Sc., F.Z.S., F.E.S., *Imperial Bureau of Entomology,* 41, *Queen's Gate, London, S.W.* 7.

Orig. Newstead, Prof. R., M.Sc., F.R.S., A.L.S., *School of Tropical Medicine, The University, Liverpool.*

1920 Nowell, W., *Department of Agriculture, Trinidad, West Indies.*

1920 Oliver, Prof. F. W., M.A., D.Sc., F.L.S., F.R.S., *University College, Gower Street, London, W.C.* 1.

1910 Osborn, Prof. T. G. B., M.A., M.Sc., *The University, Adelaide, S. Australia.*

1919 Paine, S. G., D.Sc., F.I.C., *Imperial College of Science, London, S.W.* 7.

1920 Palmer, Ray, *Ingleholme, Norton Way, S. Letchworth.*

1920 Pammel, Prof. L. H., Ph.D., *Agricultural College, Ames, Iowa, U.S.A.*

1920 Parker, T., *The Cedars Laboratories, Sheen Lane, Mortlake, London, S.W.* 14.

1914 Pearson, J., D.Sc., *Director, The Museum, Colombo, Ceylon.*

1920 Percival, Prof. J., M.A., F.L.S., *University College, Reading.*

1914 Petherbridge, F. R., M.A., *Sidney Sussex College, Cambridge.*

Orig. Pethybridge, G. H., Ph.D., B.Sc., *Royal College of Science, Dublin, Ireland.*

1914 Pole-Evans, I. B. P., C.M.G., D.Sc., M.A., F.L.S., *Division of Botany, Pretoria, Union of South Africa.*

1919 Pomeroy, A. W. J., *Govt. Entomologist, Ibadan, Southern Nigeria.*

1915 Porter, Dr Annie, *Zoological Department, S. African Institute for Medical Research, Johannesburg, Union of South Africa.*

1907 Poulton, Prof. E. B., M.A., D.Sc., LL.D., F.R.S., F.L.S., F.G.S., F.Z.S., F.E.S., *Wykeham House, Banbury Road, Oxford.*

1919 Prain, Sir David, Lt.-Col., I.M.S., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., *Royal Botanic Gardens, Kew, Surrey.*

1908 Priestley, Prof. J. H., D.S.O., B.Sc., *The University, Leeds.*

1919 Rayner, Dr M. C. (Mrs Neilson Jones), *University of London Club*, 21, *Gower Street, W.C.* 1.

1914. Rennie, J., D.Sc., F.R.S.E., 60, *Desswood Place, Aberdeen.*

1910 Rettie, T., D.Sc., 12, *Ann Street, Edinburgh.*

1921 Richards, E. H., B.Sc., F.I.C., *Rothamsted Experimental Station, Harpenden, Herts.*

1921 Roach, W. A., B.Sc., A.R.C.S., D.I.C., A.I.C., *Rothamsted Experimental Station, Harpenden, Herts.*

1914 Roberts, A. W. Rymer, M.A., F.E.S., *School of Zoology, Cambridge.*

1916 Robinson, Wilfred, D.Sc., *Department of Botany, University of Manchester.*

1918 Robson, Robert, *Institute of Agriculture, Chelmsford.*

1920 Roebuck, A., N.D.A., *Harper Adams College, Newport, Salop.*

1907 Rogers, A. G. L., *Ministry of Agriculture*, 10, *Whitehall Place, London, S.W.* 1.

1920 Russell, E. J., D.Sc., F.C.S., F.R.S., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Salisbury, E. J., D.Sc., F.L.S., *Botanical Department, University College, London, W.C.* 1.

1914 Salmon, E. S., F.L.S., *S.E. Agricultural College, Wye, Kent.*

1920 Sandon, H., B.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1921 Sargent, R. H., *Technical College, Darlington.*

1920 Searle, G. O., B.Sc., *Linen Industry Research Association, Glenmore Road, Lambeg, Belfast.*

Orig. Shipley, Sir A. E., G.B.E., M.A., D.Sc., F.R.S., *Christ's College, Cambridge.*

1919 Small, Prof. J., D.Sc., Ph.C., F.L.S., *Botany School, Queen's University, Belfast.*

1914 Small, W., M.B.E., M.A., B.Sc., *Department of Agriculture, Kampala, Uganda.*

1920 Smith, A. Malins, M.A., F.L.S., *Biology Department, Technical College, Bradford.*

1920 Smith, E. Holmes, B.Sc., *Botany School, The University, Manchester.*

1920 Smith, K. M., A.R.C.S., D.I.C., *The University, Manchester.*

1919 Smith, W. G., B.Sc., Ph.D., *College of Agriculture, Edinburgh.*

1913 South, F. W., *Agricultural Department, Kuala Lumpur, Federated Malay States.*

1919 Speyer, E. R., M.A., *Research and Experimental Station, Cheshunt, Herts.*

1920 Spinks, G. T., M.A., *Agricultural and Horticultural Research Station, Long Ashton, Bristol.*

1920 Stapledon, Prof. R. G., M.A., *Agricultural Buildings, Alexandra Road, Aberystwyth.*

1920 Stenhouse Williams, Prof. R., M.B., C.M., B.Sc., D.P.H., *Research Institute in Dairying, University College, Reading.*

1921 Stenton, R., *Pathological Laboratory, Ministry of Agriculture, Milton Road, Harpenden, Herts.*

1922 Stirrup, —, M.Sc., *Midland Agricultural College, Sutton Bonington, Loughborough.*

1919 Stone, H., *Forestry School, University of Cambridge.*

1919 Sutherland, G. K., M.A., D.Sc., 10, *Bank Parade, Preston.*

1921 Sutton, E. C. F., *Sidmouth Grange, Earley, Nr. Reading.*

1920 Sutton, Martin H., F.L.S., *Erlegh Park, Whiteknights, Reading.*

1905 Swanton, E. W., A.L.S., *Educational Museum, Haslemere, Surrey.*

1920 Tabor, R. J., B.Sc., F.L.S., *Botany Department, Imperial College of Science, London.*

1921 Tattersfield, F., B.Sc., F.I.C., *Rothamsted Experimental Station, Harpenden, Herts.*

1913 Taylor, F. H., *Dalmally Station, via Roma, Queensland.*

1915 Taylor, H. V., M.B.E., A.R.C.S., B.Sc., *Ministry of Agriculture,* 10, *Whitehall Place, London, S.W.* 1.

1919 Thomas, Miss E. N. Miles, D.Sc., F.L.S., 8, *Inglewood Mansions, West End Lane, London, N.W.* 6.

1921 Thornley, A., M.A., F.L.S., F.E.S., F.R.H.S., F.R.Met.Soc., *County Education Office, Shire Hall, Nottingham.*

1920 Thornton, H. G., B.A., *Rothamsted Experimental Station, Harpenden, Herts.*

1920 Tillyard, R. J., M.A., Sc.D., D.Sc., F.E.S., *Cawthron Institute, Nelson, New Zealand.*

1915 Treherne, R. C., *Department of Agriculture, Ottawa, Canada.*

1919 Trow, Principal A. H., D.Sc., F.L.S., *University College, Cardiff.*

1913 Urich, F. W., *Board of Agriculture, Port of Spain, Trinidad.*

1921 Voelcker, J. A., M.A., B.Sc., Ph.D., F.I.C., 1, *Tudor Street, London, E.C.*

1920 Wadsworth, R. V., *Cadbury Bros., Ltd., Research Laboratory, Bournville, Birmingham.*

1920 Wager, H. H., D.Sc., F.R.S., F.L.S., "*Hendre,*" *Horsforth Lane, Far Headingley, Leeds.*

1920 Wakefield, Miss E. M., M.A., F.L.S., *Royal Botanic Gardens, Kew, Surrey.*

1905 Walker, A. D., *Ulcombe Place, Nr. Maidstone, Kent.*

1914 Wallace, Prof. R., *The University, Edinburgh.*

1920 Waller, John Claude, B.A., *S.E. Agricultural College, Wye, Kent.*

Orig. Warburton, Cecil, M.A., *Yew Garth, Grantchester, Cambridge.*

1913 Wardle, R. A., M.Sc., *Zoological Department, The University, Manchester.*

1920 Ware, W. M., B.Sc., *Brookfield, Fremington, Barnstaple, N. Devon.*

1922 Warington, Miss K., B.Sc., *Rothamsted Experimental Station, Harpenden, Herts.*

1914 Waterston, J., M.A., D.Sc., *Nat. Hist. Museum, South Kensington, S.W.* 7.

1920 Watt, A. S., B.A., *Forestry Department, The University, Aberdeen.*

1920 Weiss, Prof. F. E., D.Sc., F.R.S., F.L.S., *Botany School, The University, Manchester.*

1905 Wellcome, Henry S., *Snow Hill Buildings, E.C.* 1.

1918 West, Cyril, D.Sc., A.R.C.S., D.I.C., F.L.S., 7, *Colfe Road, Forest Hill, S.E.* 23.

1921 Whitehead, T., A.R.C.S., *University College of North Wales, Bangor.*

1912 Williams, C. B., B.A., 20, *Slatey Road, Birkenhead.*

1909 Williamson, H. C., M.A., D.Sc., *Fishery Board of Scotland, Aberdeen.*

1914 Williamson, Capt. K. B., *King's College for Women, Household Social Science Department, Campden Hill Road, London, W.* 8.

1919 Willis, J. C., M.A., Sc.D., F.R.S., F.L.S., *Beechcroft, Clarendon Road, Cambridge.*

1920 Wilson, G. Fox, *R.H.S. Gardens, Wisley, Ripley, Surrey.*

1914 Wilson, Malcolm, D.Sc., A.R.C.Sc., *Royal Botanic Garden, Edinburgh.*

1921 Wiltshire, S. P., B.A., B.Sc., *Agricultural and Horticultural Research Station, Long Ashton, Bristol.*

1921 Woodcock, G. S., *Carlton Chemical Works, Glengall Road, Millwall, London, E.* 14.

1914 Wormald, H., D.Sc., A.R.C.S., *S.E. Agricultural College, Wye, Kent.*

1920 Wortley, E. J., F.I.C., M.B.E., *Director of Agriculture, Zomba, Nyasaland.*

1916 Wright, Herbert, A.R.C.S., *Mincing Lane House, E.C.* 3.

LAWS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

1. The Association shall be named "The Association of Economic Biologists."

2. The objects of the Association shall be to promote the study and advancement of all branches of Biology with particular reference to their applied aspects.

3. The Association shall consist of Honorary and Ordinary Members.

4. Each candidate for ordinary membership shall be nominated by two members. Such nomination shall be approved by the Council and confirmed by a vote of two-thirds of the members present and voting at the next General Meeting.

Every member elected shall receive notice from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until membership be forfeited under the Laws.

Ordinary Members shall pay an annual subscription of Twenty-five Shillings, due on January 1st of each year, or may compound for their subscription with a sum of Twenty-five Pounds.

All Ordinary Members on first election shall pay an entrance fee of half-a-guinea.

5. Ordinary Members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions and to receive a copy of the Association's publications.

Each member shall be entitled to introduce personally non-members to the Association's meetings.

6. Honorary Members shall be persons, *not subjects of the British Crown*, who have contributed in an eminent degree to the advancement of the science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members.

The number of Honorary Members shall not at any time exceed *twelve* and not more than *two* shall be elected in any one year.

Honorary Members shall not be liable to any payments and shall each receive a copy of the Association's publications.

Their privileges shall be the same as those of Ordinary Members, but they shall not be entitled to vote at the meetings.

7. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to terminate the membership of any member whose subscription shall be one year or more in arrears, or whose membership shall, from any other cause, be undesirable. No member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

8. All meetings shall be announced by circular addressed to all Members resident in the United Kingdom. The place and time of the meetings shall be decided by the Council; ten shall be a quorum at such a meeting.

9. An Annual General Meeting shall be held and shall ordinarily be the General Meeting falling nearest to the end of the year or as the Council shall decide.

At this meeting the order of business shall be:

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of members.
5. The election of Officers and other members of the Council.
6. Other business.

10. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, the Editors and twelve Ordinary Members. Two members of Council shall be designated to act as Vice-Presidents.

11. The Council shall select to retire from office at the Annual General Meeting such number of its Ordinary Members as will cause four vacancies and no member so selected for retirement, or otherwise vacating office, shall be eligible for reappointment to the Council as an Ordinary Member until after the lapse of twelve months. A list, containing the names of all members of Council who retire, and of those other members of the Association proposed by the Council to replace them, shall be sent to all members resident in the United Kingdom at least four weeks before the date of the Annual General Meeting. Any two members shall be at liberty to transmit to one of the Secretaries not less than fourteen days before the Annual General Meeting an intimation signed by them both of their desire to add the name of a member of the Association to the list of Candidates for election to the Council.

The Secretaries shall then when necessary issue to every Member resident in the United Kingdom a completed list of the Nominations out of which the Association at the Annual General Meeting shall select the names of Members appointed to fill the vacancies on the Council. In this completed list of Nominations will be stated the names of the Members proposing Candidates other than those proposed by the Council.

At the Annual General Meeting each Member present will receive a list of the names arranged alphabetically of all the Candidates proposed, and each Member who votes shall hand in person to one of the Secretaries a copy of this list on which has been indicated the names of those Candidates whom the Member voting desires to serve on the Council in place of those vacating office.

When the ballot has been declared closed the Chairman shall appoint from among the Members present two members of the Association not being Candidates for election to serve as Scrutineers. In examining the lists so handed in the Scrutineers will set aside and take no account of any ballot-paper which supports Candidates for more than the number of vacancies, and any ballot-paper which indicates the identity of the Member voting.

The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny and the Chairman before the close of the meeting shall announce the result of the ballot. In the case of an equality of votes for any Candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised by him before he announces the result of the ballot.

The Council shall thereupon proceed to elect from their body the officers of the Association for the ensuing year.

12. The Council may fill up any vacancy that may occur in the list of Officers and Council.

13. The Council shall meet at such times as they may determine; six members shall form a quorum.

The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorise their payment, and generally manage the affairs and administer the funds of the Association.

14. The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council and two Ordinary Members of the Association.

15. At a Council Meeting, prior to the Annual General Meeting, the Council shall appoint one or more Auditors to audit the Treasurer's Accounts.

16. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

17. No new Law shall be made nor any Law altered except on the proposition of the Council or the requisition of at least ten members addressed to the Honorary Secretaries. The new Laws or alterations of Laws shall be proposed in writing, signed by the requisitionists and delivered to one of the Honorary Secretaries a month before an Extraordinary General Meeting, which shall be called for the purpose.

Such proposed new Laws or alterations in the Laws shall be printed in the circular convening the Meeting, and sent to all members resident in the United Kingdom at least two weeks before the date of such Meeting.

No new laws, alterations or amendments shall be passed except by a two-thirds majority, when not less than fifteen members are present and voting.

VOLUME IX JUNE, 1922 No. 2

BIONOMICS OF WEEVILS OF THE GENUS *SITONA*[1] INJURIOUS TO LEGUMINOUS CROPS IN BRITAIN

BY DOROTHY J. JACKSON, F.E.S.

PART II.

SITONA HISPIDULA F., *S. SULCIFRONS* THUN AND *S. CRINITA* HERBST.

(With 5 Text-figures and Plate III.)

A. *SITONA HISPIDULA* F.

Sitona hispidula F. is widely distributed throughout Europe and America and is a recognised pest of leguminous crops in both continents. It is also recorded by Allard (1) from Western Siberia. In America its depredations appear to be only of recent date, as prior to 1876 this species was not known to occur, but in that year it was observed in New Jersey, and in 1889 its sudden spread in America was noted by Schwarz (15). It has since extended westward, and its first appearance in California was recorded by Van Dyke (20) in 1917.

In the British Isles it is common and widely distributed, and wherever present, is injurious to clover and lucerne, though the damage caused by it in this country has hitherto escaped recognition. The life-history of this species has been investigated by Wildermuth (22) in America, but in Europe only a few observations have been recorded by Brischke (4) in Western Prussia. Hitherto no account of the life-history of this species in Britain has been published.

Food-plants.

All species of clover (*Trifolium*), lucerne (*Medicago sativa*), medick (*Medicago lupulina*); rarely upon peas.

At Wye, Kent, this species was common upon lucerne in most months of the year; it also occurred on temporary clover leys, in fields of permanent pasture and amongst clover in waste places in the same locality. Only one or two specimens occurred upon peas. In the north of Scotland it is locally abundant amongst clover in the fields and by the roadsides. In Russia and America this species is principally recorded as a pest of clover and lucerne, but in Maryland, Cory (5) mentions it as attacking newly planted Lima beans. Petit (12) observes that *S. hispidula* was so numerous upon lucerne in Michigan that entire fields were destroyed by it. Wildermuth (22) considers that the larvae of this species may sometimes feed upon the roots of grass, but no confirmation of this has been obtained in the present research. Bargagli (2) mentions the occurrence of *S. hispidula* on *Galega officinalis*.

---

[1] The name *Sitona* Germ. is here adopted in place of *Sitones* Schoenh. on account of priority, Germar (9) having named this genus *Sitona* in 1824. Schoenherr (16) in 1826 and again in 1834 (17) also uses this name, but in 1840 (18) changed it to *Sitones*.

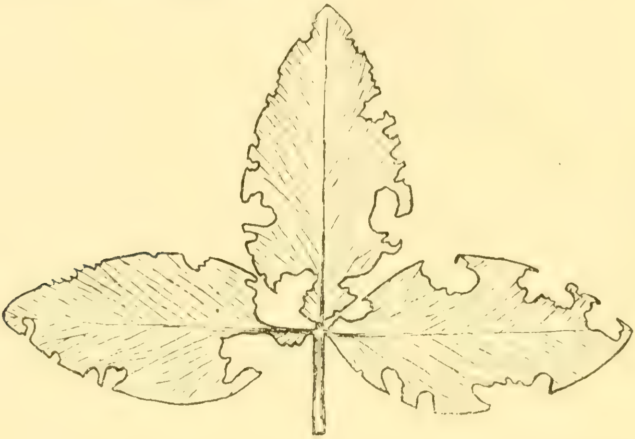


Fig. 1. Leaf of red clover showing damage by adult *S. hispidula*.

Nature of Damage.

Damage by adults. (Fig. 1.) The adults of *S. hispidula* feed upon the leaves of clover and lucerne but are rarely present in sufficient numbers to cause serious damage. They commence to feed at the edge of the leaf by biting out very small notches which are usually deepest between the veins, so that the eaten portion has often a jagged appearance. The beetles frequently continue to feed at the same place upon the leaf, thereby enlarging the original excavation and forming irregular indentations of various sizes.

Damage by larvae. (Figs. 2 and 3.) Though larvae of *Sitona* are to be found very commonly damaging roots of clover in the field it is not easy to determine to which species they belong, as at present there is no comparative description of the larvae published, and also when dug up from the field they are difficult to rear. The injury was therefore determined

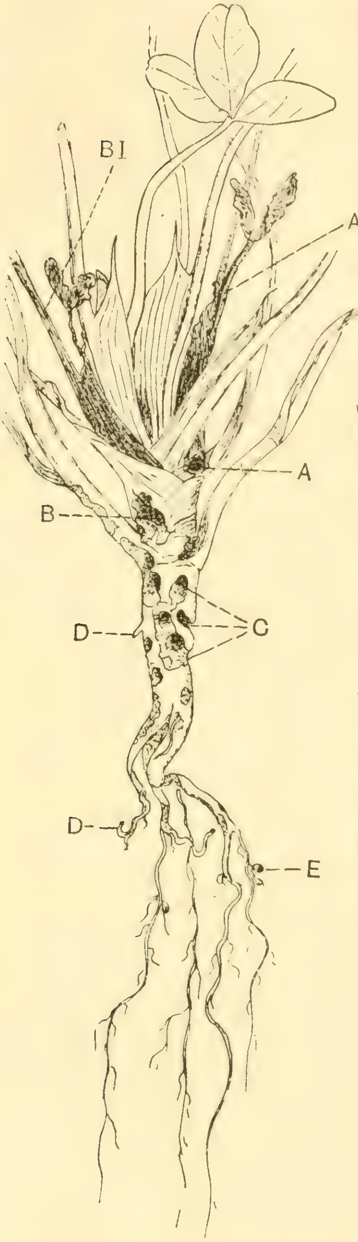


Fig. 2.

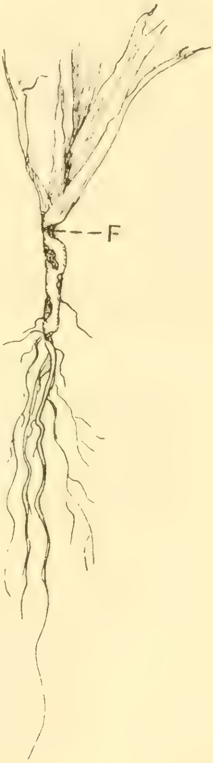


Fig. 3.

Fig. 2. Plant of red clover with root damaged by larvae of *S. hispidula*. *A* and *B* = holes at base of plant bored by larvae and causing death of shoots *A* 1 and *B* 1. *C* = holes bored on root. *D* = side rootlets eaten by larvae. *E* = root nodule eaten by larva.

Fig. 3. Young plant of red clover almost bitten through at *F* by larvae of *S. hispidula*.

by breeding experiments. Eggs were introduced into pots of clover covered with muslin (10, p. 283, Plate XVIII, fig. B). In uninfected pots the clover remained strong and healthy, but in pots infected with eggs of *S. hispidula* much of the clover died before the larvae had ceased feeding owing to the damage they had inflicted upon the roots and the plants that survived were thin and weak. The larvae bored deep holes all over the main root and when half grown they were sometimes found entirely buried in the root. The portion of the root just below the crown of the plant was frequently chosen for attack with the result that the shoot immediately above the damaged area died. In all the larger plants so affected the outer shoots were dead from this reason whilst in small plants the whole root was sometimes bitten through at this point. The surface of the main roots were also gnawed in patches, the side rootlets were bitten off and the nodules destroyed. The gnawed portions of the root decayed and turned brown.

Field observations indicate that the greater part of the injury is done in June and July when the larvae are most abundant and plants of clover with the roots injured as described above have been dug up from the fields in these months and from the larvae that were found beside them adults of *S. hispidula* were duly reared.

Description of Adult.

Black, clothed on the dorsal surface with scales of various shades of brown and ochreous and with long erect setae on the elytra. Length 3·3–4·7 mm.

Head. Eyes flat, scarcely projecting from the sides of the head. Forehead between the eyes completely flat but with a narrow central furrow which is continued upon the rostrum.

Pronotum. Broader than long, sides strongly rounded. Covered with large diffuse punctures between which are smaller punctured dots, and bearing numerous short raised setae pointing forwards. Scales rather broad and closely placed, of uniform colour, but varying in different specimens; purplish brown or greyish brown. Raised setae black or white. Broad subdorsal bands and a narrow interrupted dorsal line composed of bright ochreous or whitish scales are usually present. The anterior coxal cavities separated from the presternal line by an area equal to the breadth of the presternum[1] (Fig. 5).

---

[1] Reitter (13) in his key to the genus *Sitona* makes use of the character afforded by the position of the anterior coxal cavities in regard to the transverse furrow behind the anterior edge of the prosternum. This furrow which he calls "die Abschnürungslinie hinter dem Vorderrande der Vorderbrust," I here designate as the presternal line and the area between it and the anterior margin as the presternum.

Elytra. Rather broad and short with striae of large punctures and with conspicuous raised setae, black and white, pointing backwards and arranged in lines. These setae are as long or slightly longer than the breadth of an interval between two striae. Scales much variegated in colour in the same specimen, occurring in darker and lighter groups.

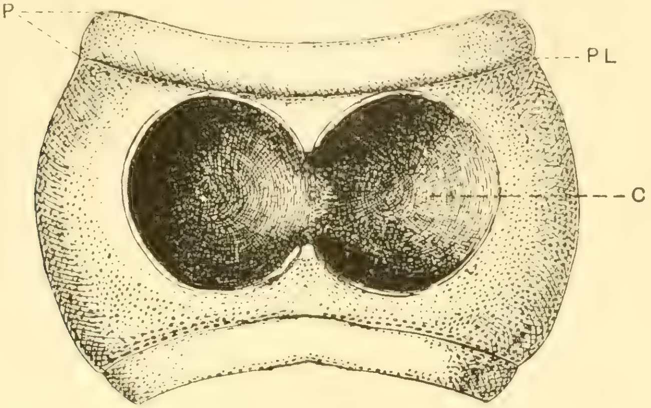


Fig. 4. Prosternum of *Sitona regensteinensis* Herbst with coxae removed. ×60. *P* = presternum. *PL* = presternal line. *C* = coxal cavity.

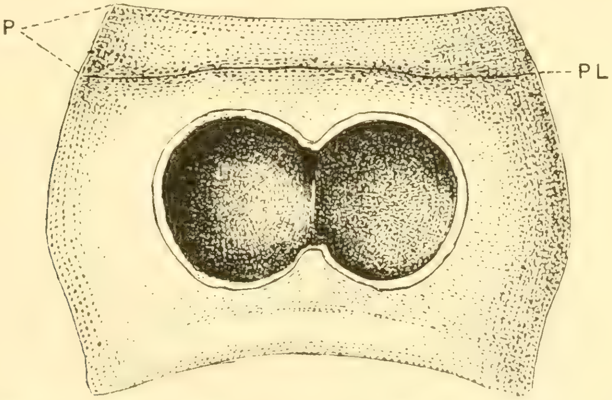


Fig. 5. Prosternum of *Sitona hispidula* F. with coxae removed. ×60. *P* = presternum, *PL* = presternal line.

Coloration varies in different specimens from dark purplish brown mottled with ochreous to pale greyish brown variegated with silvery grey. There is frequently a light patch upon the shoulders.

Undersurface. Clothed with ochreous or whitish scales and flat setae. The scales on the meso and metasternites and on the abdomen are plumate in structure.

Legs. Femora black but reddish at the base and extreme apex and bearing pale scales and long flat setae. Tibiae and tarsi red clothed with similar setae.

Antenae. Dark red with pale setae.

External Sexual Differences.

The sexes can readily be distinguished by examination of the posterior abdominal segments which are similar in structure to those of *S. lineata.*

On Distinguishing Sitona hispidula *from other British Species of* Sitona.

Owing to the difficulty usually experienced in identifying the weevils of this genus, and in order to supplement the key already given, the species which might most easily be confused with *S. hispidula* are here enumerated and some additional characters for their distinction are given.

S. tibialis Herbst and *S. lineella* Gyll. Bristles more depressed and much shorter than in *S. hispidula,* not being as long as breadth of an elytral interval.

S. crinita Herbst and *Waterhousei* Walt. Eyes prominent, projecting from the sides of the head.

S. regensteinensis Herbst. Anterior coxal cavities reaching presternal line (Fig. 4).

S. humeralis Steph. No upstanding setae. Forehead excavated between the eyes.

The Reproductive Organs of Sitona hispidula.

The reproductive organs of *S. hispidula* are similar in structure to those of *S. lineata*, but in the male differences occur in the shape of the genitalia. In the newly emerged female of *S. hispidula* the ovarian tubules are scarcely developed and the terminal chambers are very small, just as in the immature female of *S. lineata,* but unlike this species, the reproductive organs of both sexes attain full growth 6 to 8 weeks after emergence.

Alary Dimorphism[1].

In the course of dissection of *Sitona hispidula* F. it was observed that two forms of the species existed, one with fully developed wings (Plate III, fig. 1) and the other with very small vestigal wings of a peculiar shape (Figs. 2 and 3) and incapable of flight. The brachypterous wings vary in size and in distinctness of venation in different specimens, but no intermediate forms between Figs. 3 and 1 have yet been observed. The genitalia of the two forms have been examined and no differences have been detected, and moreover in captivity brachypterous males have mated with fully winged females.

---

[1] A similar case of alary dimorphism is described by Dr David Sharp in the Carabid *Pterostichus* (*Omaseus*) *minor* Gyll in *The Entomologist,* vol. 46, 82–87, 1913.

In a separate article a description will be given of the structure of the two types of wings and of the modification of the metatergum in the brachypterous form.

Up to the present brachypterous specimens have only been taken in two localities, from Wye, Kent, and in Ross-shire. In the former district fully winged specimens predominated. In Ross-shire, around Evanton, only the brachypterous form has been found[1], but further north at Kildary fully winged specimens have also been taken. The macropterous form has been taken from the following localities: Crowborough, Sussex; Brandon, Suffolk; Tring, Herts.; Haslemere, Surrey; Kingussie, Inverness-shire; Invershin, Sutherland. The distribution of the two forms appears to have no relation to latitude or altitude, nor is the short winged type rare in localities where it occurs. Thus at Swordale, Evanton, about 500 feet high the latter form is abundant, yet at Invershin about 40 miles north near sea level, and at Balavil, Kingussie at an elevation of over 700 feet, *S. hispidula* is equally common, but all the specimens so far examined have been of the long-winged type.

The Egg.

The eggs vary slightly in shape and size from 0·41 mm. by 0·37 mm. or 0·46 mm. by 0·34 mm. to 0·49 mm. by 0·34 mm. They are similar in colour and shape to those of *S. lineata*. The first laid eggs of *S. hispidula* are pointed at both ends and twice as long as broad.

The Larva.

The larva closely resembles that of *S. lineata*, but the colour of the body, especially in the immature larva, is not as white as in that species, but more translucent and greyish. Slight differences occur also in the structure of the head by means of which it is possible to separate the larvae of the two species, and it is hoped to describe these fully in a later paper. No eye spots are present. The full grown larva measures about 6 mm.

The Pupa.

The pupa is similar to that of *S. lineata* and measures about 5 mm.

---

[1] Since writing the above a single ♀ of the fully winged form has been collected in this locality.

LIFE-HISTORY.

The life-history of most of the *Sitona* which breed upon clover is complicated by the long period of egg-laying of each female, with the result that the development of the progeny of the same parent extends over a considerable time. The life-history of *S. hispidula* may be thus summarised. There is only one generation in the year. The imagines lived about 12 months. They emerged from the pupal stage from July to September and commenced to lay eggs six to eight weeks after emergence. A few eggs were laid during the winter and vigorous oviposition recommenced in spring. Towards the end of June egg-laying decreased, and during July most of the weevils died. Eggs laid late in autumn did not hatch till the following spring, but a few of the September eggs and all those laid in spring and summer hatched in 25 days. No success was obtained in rearing the few larvae which hatched in autumn from the first laid eggs, but those which hatched in the following spring and summer fed up in from 11 to 14 weeks, pupated, and emerged as adults four weeks later. The last few eggs laid by the old females in July produced full fed larvae and pupae in the end of October, but these perished during the winter. Thus it will be seen that the principal period during which the larvae occur is in the summer from the end of April until August, those larvae which hatch before the winter from the first laid eggs and those which hatch late in the following summer from the last laid eggs of the same parents being few in number and uncertain in attaining maturity. The winter is thus passed almost entirely in the egg and imaginal state.

Detailed Observations on Life-History and Habits.

The life-history has been ascertained by field observations and breeding experiments. These may be placed in three groups according as they relate (1) to the imagines, (2) to the length of the egg stage, and (3) to the length of the larval and pupal period.

I. *The Imagines.*

Length of Life and Period of Oviposition.

(*a*) *Field Observations.*

Adult *S. hispidula* were obtained from various localities at different times of the year. If sexually mature the females laid eggs readily in the boxes in which they were collected. They were never subjected to artificial temperature. If immature, the beetles were sleeved in muslin bags upon pots of clover kept out of doors and the date of oviposition observed. The results of these observations are here tabulated.

Date	Locality where collected	Condition	Remarks
1921 July 27th to Aug. 1st	Wye, Kent	Not laying eggs. Ovaries quite immature.	Fairly common.
1919 Aug. 11th to Sept. 1st	Bournemouth	Not laying eggs. Ovaries immature.	Sleeved on clover and commenced laying eggs from middle to end Sept.
1919 Sept. 4th to 8th	Sudbury, Suffolk		
1920 Sept. 16th	Invershin, Sutherland	Some laying eggs and others not, ovaries of some mature, of others immature.	Very common.
1921 Sept. 20th	Evanton, Ross-shire		
1918 Oct.	Wye	Laying eggs.	Common.
1921 Jan. 31st and Feb. 5th	Evanton	Laying a few eggs.	
1921 Mar. 13th	Haslemere, Surrey	Laying eggs.	Common.
1918 1919 April and May	Wye	,, ,,	,,
1921 April and May	Evanton	,, ,,	,,
1918 June	Wye	,, ,,	Scarce.
1921 June	Evanton	,, ,,	,,
1919 July 30th	Sudbury	Laying a few eggs. Ovaries typical of very old female.	Only one old female obtained, easily distinguishable from the new generation by condition of reproductive organs.

(*b*) *Experiments.*

In order to determine the length of life and the period of oviposition adults collected at Wye in October 1918 and laying eggs were sleeved on clover growing in pots out of doors. On January 27th, during deep snow, some of the beetles were removed to a glass dish which was left in one of the pots, and two or three eggs were laid in it, but many of the beetles died, doubtless owing to insufficient protection from the severe frost. During the winter many of the remaining weevils in the pots died, but those that survived laid eggs in April and throughout May. By the end of May some had ceased oviposition and by June 22nd only a few individuals were still laying eggs, and still fewer in the beginning of July. By the middle of the month most of them had died, though one or two survived till August.

Period of emergence of imagines. This was determined by breeding experiments to be described later in which the weevils emerged during August and September. When sleeved on clover they commenced oviposition on October 22nd, about a month later than the specimens in the field, but similar experiments tend to show that *Sitona* take longer to develop when bred in captivity.

Habits.

During fine weather in September and October the beetles are most abundant and may be obtained by sweeping clover or lucerne. They are very active on sunny days at this time of the year and are frequently to be met with on palings or stone walls. It is probable that the principal migration to the new fields occurs at this time. At Invershin, Sutherland, from September 16th to 21st, 1920, several hundred beetles of this species were observed upon the walls of a wooden building adjoining fields of grass and hay. On sunny afternoons numbers were seen crawling up from the ground, but despite careful watching I failed to observe any specimens flying on to the wall, although all the specimens examined from this locality proved to have fully developed wings. The weevils dropped down from the woodwork at the slightest touch. They were equally common on the walls in the shade as in the sun, but were rare on the stonework of the house. Many got in at the windows. *Sitona sulcifrons*, a very common species with brachypterous wings also occurred upon the walls but in much fewer numbers. The following year on revisiting this district on a fine day on September 21st no such swarms were observed, though the weevils were very common on the clover. This activity of the imagines in autumn is not confined to the winged individuals, but has also been observed in brachypterous specimens at Swordale, Ross-shire. In France, Bedel (3) has made some interesting observations upon a migration of *Sitona gemellata* in the end of September and beginning of October.

In the winter the adults of the *S. hispidula* continue to feed, and even during continuous frost in January and February, they were to be found in the fields in Ross-shire lying on the surface of the ground beneath freshly eaten clover leaves. On sunny days in March and April the beetles may be seen walking on the clover leaves but are more frequently taken at the base of the plant. They are also active at night. They lay their eggs indiscriminately wherever they happen to be resting.

Number of eggs laid. The number of eggs laid by a single female from the commencement to the end of oviposition has not been ascertained, but the following experiments carried out in the laboratory show the number of eggs laid by two females after hibernation. Artificial conditions are doubtless responsible for the greatly prolonged life of the female in the first experiment. This has happened with other *Sitona* kept indoors and regularly fed, and no corroboration of such a prolonged life has been obtained by field observation or outdoor experiments.

		Apr. 14th to 30th 1919	May	June	July	Aug.	Sept. and Oct.	Nov.	Dec. to end Apr. 1920	May and June 1920	Total
1st ♀	No. of eggs laid	43	193	171	159	106	120	27	27	77	924
2nd ♀	,, ,,	63	148	Oviposition ceased							211

As a rule from two to five eggs are laid daily, but as many as twelve per day have been observed.

II. *Length of the Egg Stage.*

In order to determine the length of the egg stage, eggs laid between certain dates were placed on damp earth in small dishes and the latter kept in tins containing a layer of wet moss, the tins being kept out of doors or in an unheated room. The result of these observations may be tabulated as follows:

Locality from which parent beetles were obtained	Date of oviposition	Date of hatching
Bournemouth	Sept. 17th to Oct. 8	A few larvae only on Oct. 12th.
Invershin	Sept. 22nd to Oct. 13	Oct. 31st to Nov. 2nd three larvae only. Feb. 16th to April 25th, 42 larvae, the majority hatching in March.
Wye	Oct. 13th to 16th	Two larvae only on Nov. 18th.
Sudbury	Oct. 16th to 31st	Mar. 27th to Apr. 12th.
Evanton	May 6th to 10th	Commenced May 31st.

III. *Length of Larval and Pupal Stages.*

The time occupied in the development of larvae resulting from eggs laid at different times of the year has been ascertained by means of the following breeding experiments, in which, except when otherwise stated, the eggs were placed at the roots of clover growing in sleeved pots out of doors. It was necessary to duplicate many of these experiments to observe the habits and development of the larvae, owing to the disturbance of the soil this involved. The length of the egg stage being already known and the pupal stage lasting about four weeks the length of the larval stage can be deduced when the imagines emerge by subtraction of these periods.

1. *Development of the Autumn-laid eggs.*

(*a*) *The Autumn Hatching Larvae.*

Eggs laid September 17th to October 8th, 1919 produced a few newly-hatched larvae on October 12th. Eggs and larvae placed in pot on

October 12th. *December 5th*—soil of pot thoroughly searched but no larvae found.

(*b*) *The Spring Hatching Larvae.*

Eggs laid	Results
Sept. 17th to Oct. 18th, 1919	*Aug. 31st*, 1920. Imagines emerged.
Oct. and Nov., 1919	*June 2nd*, 1920. Larvae 2·2–3·3 mm. long occurring 1 or 2 inches below the surface soil, close to the small fibrous roots, and destroying the root nodules.
,, ,,	*July 3rd*, 1920. Larvae half to full grown. Injuring main root of clover.
,, ,,	*July, end.* Pupae.
,, ,,	*Aug. 19th.* Imagines.
	LARVAL PERIOD. 15–16 weeks.

2. *Development of the Spring and Summer Laid Eggs.*

Eggs laid	Results
Apr. 14th to 24th, 1919[1]	*June 14th.* Larvae 1·6 mm. long to 2·7 mm.
,, ,,	*July 9th.* Larvae 2·5–3·5 mm.
,, ,,	*July 28th.* Fully grown larvae.
,, ,,	*Aug. 12th.* Pupae.
,, ,,	*Aug. 26th to Sept. 1st.* Imagines.
	LARVAL PERIOD. About 11 weeks.
May, 1919	*Sept. 17th.* Imagines.
June 21st to July 21st[1]	*Oct. 31st.* Full grown larvae. Pupae. *Nov. 28th.* Pupae. Failed to rear adults.

The above experiments have been carried out in Ross-shire. It is probable that in the south of England the larvae develop more rapidly, as in fields at Wye, Kent, full grown larvae and pupae of this species were found on June 15th, from which imagines were reared from the beginning to the 21st July. From the field in Ross-shire pupae were obtained from July 5th to August 11th and adults reared from them from the end of July to the end of August. It was noticed that when larvae were reared in Ross-shire in a glass-house, the temperature being raised by the sun alone, the larval period occupied only eight weeks. During the winter and early spring repeated search was made for larvae of this species both in the south of England and the north of Scotland in localities where the adult was common, but always without success; *Sitona puncticollis* and *S. flavescens* being the only species found in the larval stage at that time.

Life-History in America.

In America, according to Wildermuth (22) the life cycle of this species occupies a very much shorter time. Thus the egg stage lasts 13 days, the larval period 17–21 days and the pupal stage 8–10 days. Only one generation has been observed in the year.

---

[1] These eggs were placed at the roots of clover previously planted out of doors in one of the large breeding cages already described (10, pp. 283–284, Plate XIX).

NATURAL ENEMIES.

In America (6, 11) many birds have been observed feeding on the adults.

Insect Parasites.

No insect parasites have hitherto been recorded from *Sitona hispidula*, but in the course of this research three Braconids[1], *Perilitus rutilus* Nees, *Perilitus aethiops* Nees and *Pygostolus falcatus* Nees (the dark variety described by Ruthe) have been bred from the adult beetles. Single Hymenopterous larvae have been found on several occasions within the body of the beetles.

Protozoan Parasites.

Gregarines have frequently been observed in the alimentary canal of adult *Sitona hispidula* and also of *S. puncticollis*. Dr H. M. Woodcock has most kindly examined those from the latter species and has identified them as belonging to the genus *Gregarina*. Those from *S. hispidula* appear to be the same.

Fungus Parasite.

The fungus, *Botrytis bassiana* (Balsamo) Montagne appears to be the most serious natural enemy of this species and attacks both adults and larvae.

B. *SITONA SULCIFRONS* THUN.

Sitona sulcifrons is recorded by Reitter (13) as occurring throughout Europe and in the Caucasus and its injuries to various leguminous crops have been observed in France, Germany and Russia. In the British Isles it is widely distributed and is often exceedingly common upon red clover, especially in the north of Scotland. So far as I am aware, no observations have been recorded regarding the life-history of this species or the habits of the larvae.

Food-plants.

All species of clover (*Trifolium*) also bird's-foot trefoil, *Lotus corniculatus*. In France, Girard (24) records this species as damaging peas, and Allard (1) mentions its abundance on lucerne. According to Rushkovsky (26) peas, clover, lucerne and buckwheat are attacked by it in Russia. Rye (27) records the abundance of this species on lucerne on the south coast of England, but this I have not yet been able to corroborate.

---

[1] I am much indebted to Mr G. T. Lyle for his identification of these and other Braconids mentioned in this paper.

Nature of Damage.

Damage by Adult. The adults of *Sitona sulcifrons* feed upon the leaves of clover in the same way as those of *Sitona hispidula.* As a rule, however, the eaten areas are more regular than in that species and more or less U-shaped. From July to October nearly every clover leaf in certain fields of first and second year "seeds" in Ross-shire showed the characteristic notches eaten by this species, but the damage was never sufficient to check the growth of the plant. The adults could often be swept from the clover in numbers in this locality and out-numbered those of any other species of *Sitona.* In Kent, *S. sulcifrons* appears to be less generally distributed, but was abundant in temporary clover lays on the Downs at Wye, though rare at a lower elevation.

Damage by larvae. The larvae appear to feed principally upon the root nodules of the clover and they sometimes damage the small fibrous roots which bear them. Unlike the larvae of *S. hispidula* they have never been observed attacking the main root. The larvae occur in the soil to a depth of about 2 inches.

Description of Adult.

Black, sparingly covered with copper coloured scales and flat setae which are frequently abraded. Size 2·9 to 4·2 mm.

Head. Eyes prominent projecting from the sides of the head and with their dorsal edge higher than the level of the central furrow which runs down the middle of the forehead to the rostrum. The forehead between the eyes is not flat but gradually slopes downwards from the eyes on each side to meet the central furrow. Punctuation and scales very similar to pronotum.

Pronotum. Broader than long, covered with fairly closely placed punctures which, though comparatively large, are shallow. Sparingly clothed with flat copper coloured or ochreous setae, resembling scales but hair-like in width, and with indications of lighter dorsal and subdorsal lines composed of similar but more closely placed setae interspersed with elongated scales of pale yellow or copper. Anterior coxal cavities just reaching presternal line.

Elytra. Rather broad and short. Punctured striae most conspicuous anteriorly but becoming obsolete towards the apex. Individual punctures comparatively large. Intervals with finely punctured dots. Sparingly covered with elongated, usually copper coloured scales interspersed, especially on the sides, with flat setae of the same colour. Pale yellow or silvery scales occur in groups producing a variegated effect.

Sides and under-surface. A broad stripe of large pale scales extends along the sides of the thoracic segments commencing behind the eyes. The scales on the posterior portion of this band and also those on the ventral surface of the thorax are plumate in structure. Abdominal sternites covered with long whitish flat setae and a few plumate scales.

Legs. Femora black with pale flat setae and a few scales; tibiae and tarsi light red with similar setae.

Antennae. Light ferruginous, with pale setae, club darker.

External Sexual Differences.

The posterior abdominal segments differ in structure according to the sex as in *Sitona lineata.*

Species liable to be confused with S. sulcifrons *and characters which distinguish them.*

S. suturalis Steph. Eyes depressed and not projecting dorsally from the level of the forehead.

S. lineata L. Forehead, though with central furrow, quite flat between the eyes.

S. humeralis Steph., *S. puncticollis* Steph., *S. flavescens* Marsh and *S. cylindricollis* Fahraeus. Anterior coxal cavities not reaching presternal line.

S. sulcifrons is not likely to be confused with the bristle-bearing species of *Sitona.*

Wings of S. sulcifrons. (Plate III, Figs. 4 and 5.)

Specimens of *sulcifrons* collected from various parts of England and Scotland have proved on examination to have brachypterous wings (Fig. 5). These, however, are totally different in shape to those of *S. hispidula.* They are of nearly equal breadth throughout and are evenly rounded at the apex. They measure from 1·28 mm. long by 0·38 mm. broad to 1·44 mm. long by 0·49 mm. broad. The wings show even less trace of venation than those of *S. hispidula,* only a small portion of the costal and sub-costal nervures at the base of the wing being discernible. The wings are extremely delicate and often to be found folded irregularly into a narrow strip beneath the elytra.

A curious variation in the shape of the wings has been observed in a specimen collected at Invershin, Sutherland. In this (Fig. 4), the wing is very long and narrow, measuring 1·6 mm. long by 0·34 mm. broad and is narrowed towards the apex.

In relation to the vestigial character of the wings of *S. sulcifrons*, it is interesting to note that this species is often more abundant throughout the clover in first and second year "seeds" than any winged *Sitona* of the same habits, showing that impossibility of flight is no check to the local dispersal of the species. More information is required as to how much such winged species as *S. flavescens*, *puncticollis* and *hispidula* fly, and any observations on this point would be gratefully received. These species are very active upon their legs, but up to the present I have rarely observed any of them on the wing, and it seems probable that in many cases they may migrate to new crops in the same manner as *S. sulcifrons*.

The Egg.

The egg is similar to that of *S. lineata*. It varies slightly in size and shape from 0·37 mm. by 0·27 mm. to 0·41 mm. by 0·31 mm.

The Larva.

The larva is very like that of *S. lineata* and measures when full grown about 4·9 mm.

The Pupa.

The pupa measures from 3·2 mm. to 4·9 mm.

LIFE-HISTORY.

Sitona sulcifrons is a smaller species than *S. hispidula* and the egg, larval and pupal stages are all a little shorter. The reproductive organs of the adult also mature more quickly. Otherwise the life-history of this species much resembles that of *S. hispidula* and has been determined by similar breeding experiments and field observations which will be summarised as briefly as possible.

I. *The Imagines.*

Length of life and period of Oviposition. Newly emerged specimens, sexually immature, were taken in Suffolk in the end of July, and in Ross-shire on August 10th. Sleeved upon clover, oviposition commenced on September 12th and 25th respectively, and this was corroborated by field observations. Placed in pots of clover covered with muslin out of doors in Ross-shire, the majority survived the winter, and some lived until the following August. In the field, the beetles continue to feed during the winter, and even lay a few eggs. In April and May oviposition recommences vigorously, but few eggs are laid in June and still fewer in

July. In August, specimens of the old generation are rare in the field. The habits of the adults are similar to those of *S. hispidula*.

Emergence. In the breeding experiments the weevils emerged during August and September and commenced oviposition on September 17th.

II. *Length of Egg Stage.*

Locality from which parents were obtained	Date of oviposition	Date of hatching
Sudbury, Suffolk	Sept. 12th to Oct. 1st	Mar. 18th to 30th
Wye, Kent	Oct. 13th	Mar. 22nd to 27th
Evanton, Ross-shire	Nov. 19th to 27th	Apr. 2nd to 12th
Wye	Dec. 15th to 24th	Apr. 3rd to 24th
,,	May 27th to June 2nd	June 19th to 25th

From the above it will be seen that unlike *S. hispidula* none of the eggs laid in autumn by the newly emerged beetles hatched till the following spring.

III. *Length of Larval and Pupal Stages.*

The pupae occur in cells about ½ inch below the surface of the earth. The pupal stage lasts about 24 days. The larvae developed as follows:

1. *From Autumn Laid Eggs.*

Locality where experiment was carried on	Date of oviposition	Results
In pots and large breeding cages in Ross-shire	Oct. and Nov., 1918	May 5th, 1919. Larvae 1·35 mm. long.
	,, ,,	June 30th. Some larvae full-grown, one pupa.
	,, ,,	July 17th to Aug. 25th. Emergence of imagines.
		Larval period. About 13 weeks.
In breeding cage at Wye, Kent	Oct. 13th to 30th, 1918	July 5. Full-grown larva and pupa.

2. *From Spring and Summer Laid Eggs.*

Locality	Date of oviposition	Results
Breeding cage, Ross-shire	Apr. 19th to May 6th, 1919	July 14th, 1919. Larvae 3–5 mm. long.
	,, ,, ,,	Aug. 19th. Full-grown larvae and pupae.
	,, ,, ,,	Aug. 23rd to beginning of Sept. Imagines.
		Larval period. About 11 weeks.
Pots, Ross-shire	May, 1919	July 28th, 1919. Small to half-grown larvae.
	,, ,,	Sept. 26th. Imagines.
,, ,,	June 6th to 13th	Nov. 23rd. Imago.
,, ,,	July 9th to 30th	Dec. 1st. 15 full-grown larvae, 10 of them dead. None survived.

In the above experiments larvae occurred from April to December. They were most common from June till the middle of August and at this time they were abundant also at roots of clover in the fields in Ross-shire. The larvae obtained in the above breeding experiments in December died during the winter. It will be seen that they were the product of eggs laid in July and under natural conditions only a very few eggs are laid at this time. No larvae of this species have been found in the fields in winter though repeated search has been made in localities where the adult is common. A small *Sitona* pupa, probably of this species was, however, obtained on January 4th and it is possible that a few individuals, resulting from eggs laid late in the summer, may pass the winter in the pupal stage. Larvae of this species obtained from the fields in July gave rise to imagines from July 30th to October 1st.

Insect Parasites.

Insect parasites of *Sitona sulcifrons* appear to be rare and none have hitherto been recorded. Two Braconids, *Perilitus cerealium* Hal. and a species of *Liophron* have, however, been bred from adult *S. sulcifrons* and single Hymenopterous larvae have occasionally been found within the body of the beetles.

Fungus Parasite.

Similar to that of *S. hispidula.*

C. *SITONA CRINITA* HERBST.

Sitona crinita is one of the principal species mentioned by Miss Ormerod (35) and Curtis (31) as attacking peas and beans in England, and for this reason it has been included in the present research. So far, however, I have not found it sufficiently abundant on any crop to cause injury, but its profusion upon tares in the south of England is recorded by Walton (37) and Rye (27) and Mr S. R. Ashby tells me that he has found it very commonly upon vetches in Kent and in Cambridgeshire. At Wye, Kent, I have found it generally distributed and sometimes common on tares, but never abundant. It frequented the same food plants as *S. lineata* but was always vastly outnumbered by that species. It is rare in Scotland. Abroad it is widely distributed, occurring according to Allard (1), Reitter (13) and Henshaw (33), throughout Europe, in Central and East Asia, North Africa and America. It is recorded in Russia (26, 32, 36) as a pest of cultivated Leguminosae, and only in that country has its life-history been investigated (32).

Food-plants. Tares (*Vicia sativa*), lucerne (*Medicago sativa*), medick (*Medicago lupulina*), sainfoin[1] (*Onobrychis sativa*) all species of clover;

---

[1] Mr P. Harwood tells me he has taken *S. crinita* in abundance on sainfoin near Newbury in August, 1907.

less commonly upon peas and beans. Mr G. Fox-Wilson informs me that he found *Sitona crinita* seriously damaging the young flowers of *Cytisus biflorus* at Wisley on October 14, 1920.

Other recorded Food-plants. Rushkovsky (26) records this species from buckwheat in Russia, and Bainbridge Fletcher (30) from indigo and senji in India.

Nature of Damage.

The weevils eat semi-circular patches from the edges of the leaves. The larvae feed upon the root nodules and when nearly full grown they also occasionally bore channels in the main root close to the surface of the ground. The young larvae up to 2 mm. in length are to be found entirely buried in the root nodules, but when larger they feed freely upon them. An infected nodule can often be recognised by one end being darker owing to the excrement accumulated in it, and by the presence of a small hole through which the larva has entered.

Description of Adult.

Black, clothed with greyish white or ochreous scales and with raised setae on pronotum and elytra. Size 3·3 to 4·5 mm.

Head. Forehead broad, eyes very prominent. A central furrow commencing opposite the middle of the eyes is continued upon the rostrum and the area on either side of this furrow is slightly excavated. Unlike *Sitona Waterhousei* Walt., the breadth of the head across the eyes is barely twice the breadth of the rostrum at the apex. Pubescence and sculpturing as in pronotum.

Pronotum. A little broader than long, but much narrower than the elytra. With large deep closely placed punctures and evenly covered with broad ochreous or whitish scales and with short bristles. Narrow dorsal and broad sub-dorsal stripes are formed by lighter and more closely placed scales. Anterior coxal cavities separated from collar line by an area as broad as presternum.

Elytra. Shoulders prominent, sides almost parallel. With striae of medium-sized punctures; intervals minutely pitted. Scales similar to pronotum. Raised setae longer and backwardly directed, brown or white. Elytra frequently mottled with brown patches composed of long lineal brown scales; anteriorly often with indications of alternate darker and lighter longitudinal stripes (formed of darker and lighter scales) which may be continued to the apex of elytra.

Under-surface. Clothed with whitish ochreous plumate scales, and, on the abdomen, also with pale setae.

Legs. Femora black, those of 2nd and 3rd pair reddish at base and apex. Covered with pale scales and flat setae. Tibiae and tarsi ferruginous with pale setae.

Antennae. Rather short, ferruginous, with club darker.

External Sexual Differences.

Posterior abdominal segments similar in form to those of *S. lineata.*

Species liable to be confused with S. crinitus *and characters which distinguish them.*

S. Waterhousei Walt. Breadth of head across eyes 2½ times the width of the rostrum at the apex.

S. regensteinensis Herbst and *S. tibialis* Herbst. Anterior coxal cavities reaching presternal line.

S. lineellus Gyll. Forehead between the eyes quite flat.

S. hispidula F. See p. 98.

Wings of S. crinita.

All the specimens of *S. crinita* so far examined have had fully developed wings.

Eggs.

The egg measures from 0·34 by 0·26 mm. to 0·36 by 0·29 mm.

Larva.

The larva measures about 5 mm.

The Pupa.

The pupa measures from 3·8 to 4·9 mm.

LIFE-HISTORY.

The life-history of *S. crinita* is closely similar to that of *S. lineata* and can be summarised as follows:

Imagines. Newly emerged specimens, sexually immature, were obtained from peas in Suffolk on August 4th. Other specimens collected in September and October from lucerne, medick and seedling tares in Kent and Suffolk were also sexually immature. Sleeved upon clover out of doors these beetles laid no eggs till the following spring when oviposition commenced in May. Specimens collected in the field in April, May and June were laying eggs. The majority of the beetles collected from the field at this time died off before the autumn of the same year, but a few individuals have lived in captivity in the laboratory for over two years.

Length of egg and larval stages. Eggs laid on 4th June commenced hatching on June 26th. To determine the larval period eggs laid in the end of May were introduced into sleeved pots of beans. From these full-grown larvae and pupae were obtained on August 17th and the adults commenced to emerge from August 27th to September 19th. They laid no eggs till the following June.

Parasites.

A Braconid belonging to the genus *Perilitus* has been bred from an adult *Sitona crinita* and single Hymenopterous larvae have been found on several occasions within the body of these beetles.

The fungus *Botrytis bassiana* Balsamo (Montagne) attacks the adult beetles.

D. SUMMARY.

1. *Sitona hispidula* is common throughout Great Britain upon clover and lucerne.

2. The adults eat the leaves and the larvae damage the roots.

3. *Sitona sulcifrons* is frequently abundant upon "seeds" clover and the larvae feed upon the root nodules.

4. *Sitona crinita* frequents tares, clover, lucerne, etc., but is rarely sufficiently common to cause injury.

5. The adults of *S. hispidula* are either fully-winged or brachypterous and two forms of brachypterous wings have been observed in *S. sulcifrons*.

6. The life-history of these three species has been investigated in Britain for the first time.

7. There is only one generation in the year.

8. The adults live 12 months.

9. The period of oviposition and the length of the egg, larval and pupal stages varies according to the species.

10. The Braconids *Perilitus rutilus* Nees, *P. cerealium* Hal., *P. aethiops* Nees, *Pygostolus falcatus* Nees and a species of *Liophron* are recorded for the first time as parasites of the adult beetles.

11. The fungus *Botrytis bassiana* (Balsamo) Montagne attacks these species of *Sitona*.

REFERENCES.

Sitona hispidula F.

(1) *ALLARD, E. (1864). Séance du 23 Mars, *Ann. Soc. Ent. France*, IV, Series 4, 329–382.

(2) *BARGAGLI (1904). *Bull. Soc. Ent. Italy*, XXXVI, 8–10.

(3) BEDEL, L. (1873). *Bull. Soc. Ent. France*, Séance de l'année 1873, cxcv.

(4) BRISCHKE, G. S. A. (1876). *Entom. Monatsblätter* 1876, 38.

(5) CORY, E. N. (1915). Abstract in *Rev. App. Ent.* A. III, 709.

(6) DEAN, G. A. (1916). "Insects injurious to Alfalfa," *Kansas State Agric. Coll., Div. of Coll. Exten., Manhattan, Bull.* No. 5, 36 pp., 39 figs.

(7) FABRICIUS, J. C. (1776). *Genera Insectorum*, 226.

(8) *FOWLER, W. W. (1891). *Coleoptera of the British Islands*, V, 216–226.

(9) GERMAR, E. F. (1824). *Insectorum Species Novae*, I, 414.

(10) JACKSON, D. J. (1920). *Ann. of App. Biol.* VII, 2 and 3, 269–298.

(11) KALMBACH, E. R. and GABRIELSON, I. N. (1921). Abstract in *Rev. App. Ent.* A. IX, 305.

(12) PETIT, R. H. (1917). Abstract in *Rev. App. Ent.* A. VI, 340.

(13) *REITTER, E. (1903). *Bestimmungs-Tabellen der europäischen Coleopteran*, LII Heft Curculionidae, 9 Theil Genus *Sitona* Germ. and *Mesagroicus* Schönh.

(14) REPPERT, R. R. (1921). Abstract in *Rev. App. Ent.* A. IX, 389.

(15) SCHWARZ, E. A. (1889). *Proc. Ent. Soc. Wash.* I, 248–249.

(16) SCHOENHERR, C. J. (1826). *Curculionidum Disposito Methodica*, 134.

(17) *—— (1834). *Genera et Species Curculionidum*, II, Part 1, 96, 123 and 124.

(18) —— (1840). *Genera et Species Curculionidum*, VI, Part 1, 253.

(19) STEPHENS, J. F. (1831). *Illustrations of British Entomology*, IV, 134.

(20) VAN DYKE, E. C. (1917). Abstract in *Rev. App. Ent.* A. V, 421.

(21) WEBSTER, F. M. (1915). *U.S. Dept. Agr. Farmers' Bull.* 649, 1–8.

(22) WILDERMUTH, V. L. (1910). *U.S. Dept. Agr. Bur. Ent. Bull.* 85, Part 3, 29–38, figs. 15–19.

Sitona sulcifrons Thun.

(23) BEDEL, L. (1873). *Bull. Soc. Ent. France*, Séance du 26 Mars 1873, l.

(24) GIRARD, M. (1880). *Bull. Soc. Ent. France*, Séance du 28 Juillet, 1880, xciii.

(25) REITTER, E. (1916). *Fauna Germanica, die Käfer des Deutschen Reiches*, V, 71.

(26) RUSHKOVSKY, I. A. (1914). Abstract in *Rev. App. Ent.* A. III, 481.

(27) RYE, E. C. (1865). *Ent. Monthly Mag.* I, 230.

(28) THUNBERG, C. P. (1798). *Museum Naturalium Academiae Upsaliensis*, A. VI, 113, No. 4.

(29) VASSILIEV, E. M. (1913). Abstract in *Rev. App. Ent.* A. I, 527.

S. crinita Herbst.

(30) BAINBRIDGE, FLETCHER (1917). *Report of the Proceedings of the Second Entomological Meeting, held at Pusa on 5th to 12th February*, 81 and 207.

(31) CURTIS, J. (1883). *Farm Insects*, 347.

(32) DOBREDEEV, A. (1915). Abstract in *Rev. App. Ent.* A. IV, 139–140.

(33) HENSHAW, S. (1885). *List of Coleoptera of America*, 136.

D. J. Jackson del.

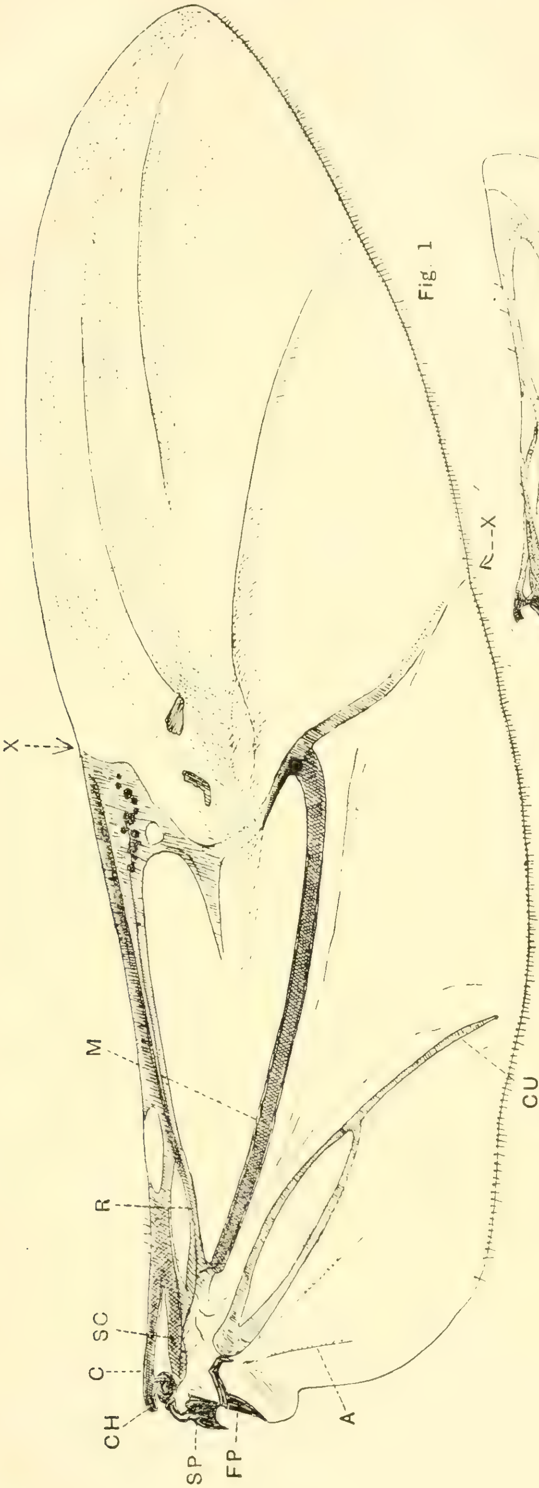

X
X
M
R
SC
C
CH
SP
FP
A
CU

Fig. 1

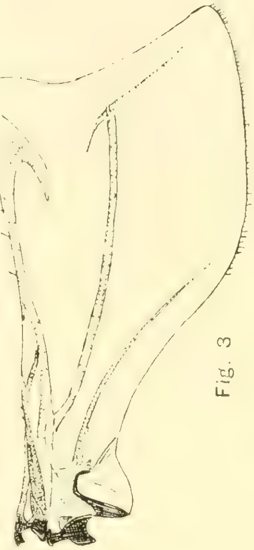


Fig. 3

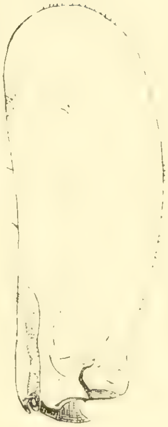


Fig. 5

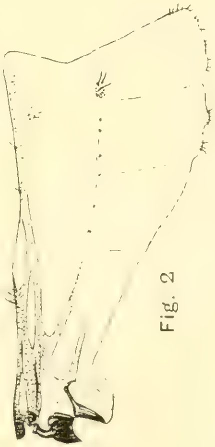


Fig. 2

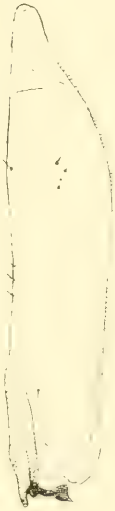


Fig. 4

(34) *HERBST, F. F. W. (1795). *Natur system aller bekannten Insekten, Der Käfer,* Part 6, 245 and 354.

(35) ORMEROD, E. A. *Reports on Injurious Insects,* 1879, 8; 1880, 5, 6; 1881, 38, 39; 1883, 57–59; 1884, 3–5; 1886, 80, 81; 1889, 15–18; 1892, 102–116.

(36) SOPOTZKO, A. A. (1916). Abstract in *Rev. App. Ent.* A. IV, 293.

(37) *WALTON, J. (1846). *Ann. and Mag. Nat. Hist.* XVII, 234.

* The references marked with an asterisk refer to works in which other species dealt with in this paper are included, and they will therefore be omitted from subsequent bibliographies.

EXPLANATION OF PLATE III

Fig. 1. Fully developed wing of *Sitona hispidula.*

Fig. 2. Brachpterous wing of *S. hispidula.*

Fig. 3. A larger form of same with clearer venation.

Fig. 4. Wing of a specimen of *S. sulcifrons* from Invershin, Sutherland.

Fig. 5. Normal wing of *S. sulcifrons.* All magnified 40 times.

A=anal; *C*=costa; *CH*=head of costa; *CU*=cubitus; *FP*=flexor plate, or 3rd axillary; *R*=radius; *SC*=subcosta; *SP*=scapular plate, or 1st axillary; *X*=point of transverse folding of wing. (Nomenclature according to A. D. Hopkins in "The genus Dendroctonus," *U.S. Dept. Agr. Bur. Ent. Technical Series,* No. 17, Part I, 1909.)

(*Received Dec. 6th,* 1921.)

"SLEEPY DISEASE" OF THE TOMATO[1]

By W. F. BEWLEY, D.Sc.

(*Director of the Experimental and Research Station, Cheshunt, Herts.*)

(With Plates IV–VII.)

CONTENTS.

	PAGE
I. Introduction	116
II. Etiology	117
1. The causal organism	117
2. Inoculation experiments	118
3. Pathological physiology	121
4. Strains of *Verticillium*	125
5. Range of hosts	125
6. Ecology	126
III. Control	129
1. Cultural methods	129
2. Elimination of sources of infection	130
IV. Summary	131

I. INTRODUCTION.

MASSEE (14, 15) first described "Sleepy Disease" of tomatoes in Britain and attributed it to *Fusarium lycopersici* (Sacc.). In the present investigation it has been found that this nomenclature covers two discrete wilt diseases in this country, *Verticillium* wilt and *Fusarium* wilt. Sleepy Disease is found throughout the British and Channel Islands where tomatoes are grown and is responsible for considerable financial losses. *Verticillium* wilt occurs more frequently than *Fusarium* wilt, which is comparatively rare. In normal years the former appears about the middle of April and increases in intensity up to the second and third weeks in May. Usually the attacks die down during the second half of June, July and August and reappear at the end of September, when the plants die prematurely. *Fusarium* wilt occurs at the hottest part of the season, usually in July and August.

Plants attacked by *Verticillium* are usually stunted, while the internodes, especially the younger, are badly developed. When the conditions

---

[1] A grant in aid of publication has been received for this communication.

of temperature and light are favourable to the fungus, the disease symptoms appear quite suddenly and the plants wilt while still green. During the night the plants may recover their turgidity, only to wilt again as the morning advances. The leaves wither from the base of the plant upwards, adventitious roots emerge from the stem and the plant dies. Death is much slower when the conditions are less favourable to the fungus: yellow blotches appear on individual leaflets on the lower leaves and these leaflets wither.

II. ETIOLOGY.

1. THE CAUSAL ORGANISM.

Since Massee described the disease as it existed in the British Isles in 1896, no further investigations have been carried out, and his views have been generally accepted in this country. He stated that the pathogen possessed two stages, the diplocladium and fusarium forms, produced from the same hyphae, but that only the fusarium stage was able to infect the plant. The present investigation is concerned with the disease of tomatoes grown under glass in the British Isles and especially in the Lea Valley. It has been found that the diplocladium and fusarium forms are not stages of the same fungus, but belong to different genera and each can, under definite conditions, produce a wilt.

In most cases *Verticillium albo-atrum* and various species of *Fusarium* may be found in the external growth at the base of a dead plant. A white growth of *Verticillium* first develops but soon becomes tinged with pink as it is overgrown by Fusaria. The almost constant appearance of *Fusarium* spores in this relation led to the idea that a species of *Fusarium* is always the cause of Sleepy Disease. During 1919–1920, 427 affected plants from different parts of England, Scotland and the Channel Isles were examined: 307 contained *Verticillium* alone, 77 contained *Verticillium* and either *Fusarium ferruginosum* or *F. sclerotioides*, 26 contained *Verticillium* and *F. oxysporum*, while 17 contained *F. lycopersici* alone. *Fusarium lycopersici* was the name given by Saccardo to a fungus which he found growing on decaying tomato fruits and has been universally applied to the species of *Fusarium* producing wilt disease of the tomato.

F. lycopersici is of comparatively little importance as a cause of tomato wilt in England, but is very destructive in America, where it is the primary cause of Sleepy Disease. Morphological and cultural studies have been made by Clayton (5), Edgerton (10), Wollenweber (20, 21) and others.

Reinke and Berthold (17) have figured and described the fungus *V. albo-atrum* as it occurs on the potato, and Carpenter (4) and Pethybridge (16) have more recently added certain details. The dominant spore form is unicellular but a considerable number of monoseptate spores are produced, especially in very old cultures, where indeed they may be the dominant form; and on certain media di- and tri-septate spores have been found.

The genus *Diplocladium* is distinguished from *Verticillium* only by having monoseptate spores, but in spite of the fact that monoseptate spores are produced in the present fungus, it is considered, that the many points of similarity to *V. albo-atrum*, in particular the fact that Reinke and Berthold originally described and figured monoseptate spores in their work, and secondly, the inoculation results obtained, entitle the *Verticillium* causing Sleepy Disease to be regarded as *V. albo-atrum*. Upon certain agar media, chiefly those to which asparagin has been added, slimy salmon-pink spore masses averaging 2 mm. by 1 mm. are produced resembling the pseudopionnotes of *Fusarium* cultures. The septate mycelium is hyaline at first but in most strains becomes brownish with age and varies from 2μ to 4μ in diameter. The mycelial cells, which give rise to microsclerotia, become swollen and by a process akin to budding a bead-like aggregate is formed, the cells of which thicken and turn brown. Radiating from the microsclerotial masses are strands of hyphae, unswollen, but thick-walled and brown. Strains which do not produce microsclerotia usually give rise to a small amount of this brown carbonised hyphae.

2. INOCULATION EXPERIMENTS.

Verticillium albo-atrum was isolated from wilted tomato plants in 1919 and tested for pathogenicity. Tomato plants, six weeks old, of the "Comet" variety were inoculated in various ways with a pure culture of the fungus. Six plants were used in each type of inoculation and six were left as controls. The stem was first washed with water, then mercuric chloride, alcohol and finally with sterile water. Small pieces of mycelium from a pure culture were pricked in and the wound covered with tinfoil. After inoculation all plants were placed in the tomato house. The controls to all experiments described in this paper were treated in precisely the same way as the inoculated plants except that no fungus was introduced.

1. *Stem Inoculations.*

(*a*) Hypocotyl. 27. iv. 19. Plants inoculated. 10. v. 19. Yellow blotches on leaf immediately above point of inoculation. 14. v. 19. First leaf wilted; second leaf above with three yellow blotches on lamina. 21. v. 19. Bottom five leaves wilted; three lowest show yellow blotches; the other two wilted but green with "leaf roll." 4. vi. 19. Plants completely wilted. Controls healthy.

(*b*) Internodes between Cotyledons and first pair of leaves. 27. iv. 19. Plants inoculated. 12. v. 19. Yellow blotches on first leaf above point of inoculation. 14. v. 19. First leaf wilted. 17. v. 19. Second leaf above stab shows a yellow blotch. 20. v. 19. Three leaves on one side and one above the other wilted; fourth shows yellow blotch. 7. vi. 19. Leaves all round the plants are wilting. 11. vi. 19. Plants completely wilted. Controls healthy.

2. *Root Inoculation.*

27. iv. 19. Three of the biggest roots per plant inoculated. 20. v. 19. First pair of leaves show yellow blotches. 29. v. 19. Lowest four leaves wilted. 19. vi. 19. Plants completely wilted. Most leaves show "leaf roll" with very little yellowing. Control plants quite healthy.

3. *Soil Inoculations.*

Sterilised soil was inoculated copiously with spores from a pure culture and placed in 6″ pots, which were then planted with plants six weeks old. 27. iv. 19. Plants inserted in pots. 17. v. 19. First three leaves wilted. 10. vi. 19. Five plants completely wilted, the remaining one with only four top leaves healthy.

Inoculations were repeated at monthly intervals and the plants reacted to infection differently according to the time of year.

Table I.

Date inoculated. 1919	No. of days after inoculation that wilt occurred	Pathological symptoms
27th May	10	Complete wilt; no yellowing
27th June	No wilt in 70	Lowest 9 leaves turned yellow and partially dried up
25th July	,,	Lowest 3 leaves desiccated
25th Aug.	53	Complete desiccation of leaves from base upwards
22nd Sept.	40	Bottom 4 leaves desiccated, remainder wilted
22nd Oct.	26	Complete wilt with practically no yellowing

The preliminary experiments proved the pathogenicity of the *Verticillium* cultures employed, and indicated that the conditions during the months of June, July, August and September are unfavourable to the rapid progress of the fungus in the plants. The hypocotyl and internode inoculations were the first to show typical disease symptoms, but the soil inoculations, although longer in producing first symptoms, produced a complete wilt as soon as the hypocotyl inoculations. Internode inoculations were slower in producing a complete wilt. Here the fungus travelled up one side of the plant first and produced a wilt on this side only. After a time the fungus worked round the stem and induced a wilt on all sides of the plant. During 1920 cultures were re-isolated from plants kept over the winter months and inoculation experiments performed to ascertain the effect of various environmental conditions upon the progress of the disease.

1920.

4. One re-isolated strain, V 33, was tested for pathogenicity and gave the first symptoms of the disease eight days after inoculation; complete wilt occurring one month after this. Further experiments were performed with this strain.

5. To ascertain the relation of the character of the plant to the progress of the disease, plants in different stages of growth and of varying degree of hardness and softness of growth were inoculated (*a*) by hypocotyl stab, (*b*) by planting in inoculated soil. The results are shown in Table II.

Table II.

Type of plant used	Mean diameter of hypocotyl in cm.	No. of plants	Age in weeks	Date of inoculation	Average no. of days between inoculation and appearance of first symptoms	Average no. of days between inoculation and total wilt
"Comet," soft growth	0·8	12	6	4. iv. 20	31	59
	1·0	12	8	4. iv. 20	21	47
	1·2	12	10	18. iv. 20	21	53
"Comet," hard growth	0·4	12	6	4. iv. 20	10	24
	0·7	12	8	4. iv. 20	8	29
	0·8	12	10	18. iv. 20	8	37
"Comet," starved growth	0·4	12	6	4. iv. 20	8	20

The observations shown in Table II indicate that plants grown hard with a thin stem, or plants obviously starved, most readily succumb to the pathogen in question. As there was very little difference in the result of the two kinds of inoculation, only those obtained by planting in infected soil are tabulated; these being the more comparable with those of plants naturally infected in the nurseries.

6. *Inoculation of sterile seedlings.*

The positive results obtained by growing young tomato plants in soil inoculated with a pure culture of *Verticillium*, does not eliminate the possibility that infection by this fungus may only be possible where the plant has been wounded previously. Under such conditions no obvious wounds may exist, but minute lesions may be present. Experiments were therefore arranged in which seedlings grown under sterile conditions were inoculated. Tomato seeds were sterilised in mercuric chloride, washed in sterile water and germinated on agar in petri-dishes, after which they were transferred to 1000 c.c. Erlenmeyer flasks in which 200 c.c. Czapek's agar with 1 per cent. saccharose had been allowed to set. The seedlings were allowed five days in which to establish themselves in the flasks and then the medium was inoculated with a pure culture of *Verticillium*. The fungus readily attacked the young roots, which it penetrated and passing into the wood caused the seedlings to wilt, on an average nine days after inoculation. Sterile untreated controls were quite healthy after 20 days. From this it seems justifiable to assume that *Verticillium albo-atrum* can infect healthy tomato plants in the absence of wounds.

7. *Fusarium* inoculations.

Four species isolated from wilted tomatoes were tested, namely, *F. lycopersici*, *F. oxysporum*, *F. ferruginosum* and *F. sclerotioides*. Inoculations were performed upon plants six weeks old of the varieties Comet, Kondine Red, Fillbasket and Ailsa Craig. Plants in different conditions of health and under various conditions of temperature, humidity and light were inoculated. *F. ferruginosum* and *F. sclerotioides* never produced wilt under any circumstances and must be regarded as saprophytes. The strains of *F. oxysporum* destroyed the pith and cortical tissues round the point of inoculation and in some cases worked into the roots, destroying the tissues as they went. In a few cases, at an average temperature of 27·8° C.–28·9° C. a slight desiccation of the lower pair of leaves was observed, but generally no wilt or desiccation resulted from inoculation with this species. *F. lycopersici* readily produced a wilt and desiccation at temperatures of 28° C.–29° C., but when the temperature was below this, infection was uncertain.

3. PATHOLOGICAL PHYSIOLOGY.

The pathological symptoms in the anatomy of plants suffering from Wilt Disease, whether caused by *V. albo-atrum* or species of *Fusarium*, are limited to a brown discoloration of the wood vessels, and the presence of fungal hyphae within them. Early writers decided that the wilting or premature death by desiccation was due to the choking of the wood vessels with fungal hyphae and Pethybridge (16) suggested the term "Hadromycosis" for these symptoms instead of the older term "Vascularmycosis."

In studying *Verticillium* wilt, Van der Lek has suggested (19) that in this disease of the cucumber the fungal hyphae enter and kill the parenchymatous tissues of the leaves. Klebahn studying the *Verticillium* wilt of dahlia (12), Haskell the *Fusarium* wilt of potatoes (11), and Brandes the *Fusarium* wilt of banana (2) have all suggested the excretion by the fungus of a toxic substance, which is carried up the vascular bundles and kills the plant. This hypothesis gains a certain amount of support from the investigations by Dixon (7, 8) and Haskell (11) on the killing of plants by various artificial means.

In the present study it was found that the walls of the vessels are stained brown and in longitudinal sections a brown gum-like substance is frequently noticed lining the lumen and blocking up the vessels. In the process of staining this is washed away, but it is readily seen in fresh material. The similarity between the colour of this gum-like substance and that of the liquids in some old culture tubes, led to an examination of culture liquids. Cultures of *Verticillium* in Dox's solution with 20 per cent. saccharose showed a distinct yellow colour in the liquid. The fungus was therefore grown in this solution for 30 days, when the liquid, which was appreciably yellow, was filtered and used in the following experiments:

A. Erlenmeyer flasks were set up with 100 c.c. respectively, of the following solutions:

Series 1. Five flasks of filtrate from cultures.
,, 2. Five flasks of filtrate heated for five minutes at 100° C.
,, 3. Five flasks of sterile medium.
,, 4. Five flasks of sterile water.

Tomato seedlings 5 inches high were cut off under water near the roots, and placed one per flask in the above series. In series 1 the plants wilted in 17 minutes; in series 2 and 3 after 2 hours; while in series 4 the plants were perfectly turgid after 24 hours. As the plants in series 3 showed wilt in 2 hours, it was evident that exosmosis was taking place, so the experiment was repeated with the liquids diluted to three times their bulk with sterile water. In this case a definite wilt was produced in series 1 in 42 minutes and slight wilting in series 2 in 95 minutes which did not increase up to 24 hours. No wilt appeared in series 3 and 4 even after 24 hours.

B. To 1000 c.c. filtrate and 1000 c.c. sterile medium respectively, absolute alcohol was added. A cloudy precipitate formed and the solutions were allowed to stand until the precipitate settled to the bottom, when the supernatant alcohol was syphoned off and the precipitate dried. After 24 hours the precipitates were dissolved in 3000 c.c. sterile water, and placed in flasks, 300 c.c. per flask.

Series 5. Five flasks with 300 c.c. each of solution of precipitate from culture filtrate.
,, 6. Five flasks with 300 c.c. each of solution as in series 5 heated for 5 minutes at 100° C.
,, 7. Five flasks with 300 c.c. each of solution of precipitate from the sterile medium.

Tomato seedlings severed from their roots were placed in these flasks as above described and a definite wilt was obtained in series 5 in 72 minutes. Series 6 yielded a slight wilt in 3 hours, but no wilt appeared in series 7 during 24 hours.

C. An attempt was made to isolate the exo- and endo-enzymes of *V. albo-atrum* by the technique devised by Brown (3), but difficulties arose in the process owing to the comparatively small size of the *Verticillium* spores. A much greater number of spores than was used by Brown had to be treated and the difficulty of obtaining a clean separation by centrifuging vitiated any attempt at quantitative determination.

The method adopted was as follows:

An abundant supply of spores was obtained by cultivating the fungus on Dox's agar with 1 per cent. saccharose, at 25° C. for 14 days, six petri-dishes of 6 inches diameter being used in each determination. The plates were covered by a thin layer of sterile water and the spores removed by gently scraping the fungal growth with a knife. Any rubbing with the finger as recommended by Brown for *Botrytis* had to be avoided, as such treatment rubbed the spores into the medium. The mixture of water, mycelium and spores was filtered through fine muslin and the filtrate consisting of water, spores and fine pieces of mycelium was centrifuged. It was almost impossible to separate much of the finer pieces of mycelium from the spores by this process, because of the lightness of the latter, but a good number of the larger pieces were removed. The spores were next germinated in strong turnip juice; 0·2 c.c. centrifuged spores being added to 21 c.c. turnip juice. Plates seven inches in diameter were each sown with 3 c.c. of the spore suspension, which was carefully spread over the surface. After 72 hours the mat of germinated spores was removed, and the turnip juice in which it had developed collected and tested for the presence of an exo-enzyme. The mat of mycelium was carefully washed to remove any spores, etc., and dried over calcium chloride in vacuo. When dry, an equal weight of clean, dry quartz sand was added, and the material ground in a mortar. The dry material was extracted for 1 hour with sterile water, using 0·2 g. mixture to 3 c.c. water. The extract was tested for the presence of an endo-enzyme capable of producing wilt, but without success.

Exo-enzyme.

The turnip juice filtrate from the germinated spores was tested in two ways. In the first instance part was de-activated by raising the temperature to 100° C. and part untreated. Seedlings 6 inches high were cut off near the base under water and placed in the active and de-activated solutions. Others were also placed in the original turnip juice as controls. All liquids caused wilt in 20 minutes owing to their high osmotic pressure. They were diluted to three times their bulk with sterile water and the experiment repeated. The active solution caused wilt in 25 minutes, the de-activated solution in 105 minutes and the original turnip juice in 5 hours. Another portion of the original filtrate was treated with absolute alcohol until no further precipitation took place. The precipitate was allowed to settle, dried over-night, and taken up with sterile distilled water the next morning; part being left untreated and part de-activated at 100° C. Seedlings cut off at the base and seedlings with roots which had been thoroughly washed in running water were placed in the solutions. Wilt was produced in the cut seedlings but not in those with roots, the active solution producing a distinct wilt in 31 minutes, and the de-activated solution in 4 hours. Similar experiments were carried out with the extract from the ground mycelium, but no wilt was observed in any case.

The above experiments were repeated several times with precisely the same results. The wilted seedlings were sectioned and examined after 24 hours' treatment. Those wilted by the active solutions showed a browning of the wood for 5 cm. up the stem. Microscopical examination showed the presence of a brown gum in parts of the wood and near the end of the stem the cambium was destroyed. The seedlings in the de-activated solutions were soft at the end, but the wood was not browned; there was no gum, and no dissolution of the cambial layers.

The above results appear to warrant the assumption that under certain conditions a definite exo-enzyme is produced by *V. albo-atrum* capable of producing wilt. Such an enzyme may act directly in virtue of its function as an enzyme, or indirectly by reason of its gum-producing powers. It can be precipitated by absolute alcohol and dried. When redissolved in water, it retains its power of producing wilt. Heating for 5 minutes at 100° C. greatly reduces its activity, but does not entirely de-activate it.

Enzyme production.

The wide range of artificial media, upon which the several strains of *V. albo-atrum* will grow indicates the probable secretion of a large number of enzymes. Modifying the methods elaborated by Crabill and Reed (6) the following specific enzymes were determined: amylase, inulase, emulsin, lipase, protease, erepsin and amidase. There was no good evidence of cytase production under the conditions tested.

4. STRAINS OF *Verticillium.*

From April 27th to May 25th, 1920, over 50 single-spore isolations of *V. albo-atrum* were made. Small pieces of diseased wood were incubated in a moist chamber and spores transferred from the fungus growth which appeared to a drop of sterile water on a coverslip. Spore dilutions were made into other drops until each drop contained one or two spores. These drops were transferred to thin layers of potato agar in a petri-dish and the position of each spore marked after examination under the microscope. As soon as germination began the spores and young hyphae were transferred to sterile tubes of prune agar. The resulting isolations were classified into six groups varying in the rate, amount and kind of growth, and in the production of colour, carbonised hyphae and micro-sclerotia, when grown in Dox's solution with 1 per cent. saccharose. All the groups have been tested for pathogenicity and there is some indication that the virulence of the strains is related to the ability to produce carbonised hyphae and microsclerotia. Group I was uniformly slow in producing the characteristic wilt, while group VI was most rapid in its effect, as is shown in Table III.

5. RANGE OF HOSTS.

The host plants used were as follows: potato (*Solanum tuberosum*), egg plant (*Solanum melongena*), snapdragon (*Antirrhinum* sp.), cucumber (*Cucumis sativus*), sycamore (*Acer* sp.), cotton (*Gossypium herbaceum*), pepper plant (*Capsicum* sp.) and elm (*Ulmus* sp.). In the first four a definite Wilt Disease and subsequent desiccation was produced. In the sycamore and cotton the plants were much stunted and the leaves withered without wilting, but in the pepper the leaves wilted and remained green. The plants were stunted in the latter case, but only a few leaves were affected.

Table III.

Group no.	Isolation no.	Per cent. of microsclerotia produced as estimated in amount of surface of medium covered	Average no. of days from inoculation to appearance of first symptoms	Mean value	Average no. of days from inoculation to complete wilt	Mean value
I	2	0	31	31·3	72	65
	39		35		56	
	43		28		67	
II	2	10	24	25	54	55
	27		26		58	
	35		25		53	
III	4	30	22	24·6	46	48
	40		25		49	
	47		27		49	
IV	1	50	20	21·6	43	45
	22		23		47	
	45		22		45	
V	3	70	26	23·3	49	51·6
	13		22		54	
	30		22		52	
VI	5	100	17	16	42	40·3
	32		19		42	
	33		12		37	

In each case *V. albo-atrum* was isolated from the diseased plants. Even after four months there was no sign of yellowing or wilting of the inoculated elms, but they were shorter than the controls and *V. albo-atrum* was isolated from the wood 2 inches above the inoculation point.

6. ECOLOGY.

Temperature.

The temperature relations of numerous fungi in pure culture have been studied in detail, but it is only within the last decade or so that the temperature factor has been related to the process of infection. This is largely the result of investigations carried out by the pathologists in the Bureau of Plant Industry in the United States Department of Agriculture and more particularly by Prof. L. R. Jones and his colleagues at the University of Wisconsin (18, 13, 9, etc.).

Such work is important especially in connection with the cultivation of crops under glass, where it is a simple matter to regulate the temperature. Observations made during the present investigation indicated an intimate relation between temperature conditions and inoculation results and showed the necessity for further inquiry into this relationship. As a series of glasshouses, where different temperatures could be maintained constantly, was not available, inoculated plants (hypocotyl stab) were placed in certain positions in the experimental houses, corridors, etc. under as different average temperature conditions as could be arranged. Twelve plants were placed in each position, and the average temperatures were calculated from readings taken twice daily from maximum and minimum thermometers placed besides the plants. The final observations, shown in the following tables, were taken 21 days after inoculation, and where figures are given each represents the average of data obtained from 12 plants.

Table IV.

	Frame	Corridor	Tomato house	Cucumber house
Average temperature ° C.	12·5	16·6	20·0	25
Absolute minimum ° C.	5·6	11·1	12·2	20·6
Absolute maximum ° C.	20·6	22·2	27·8	33·3
Date of inoculation	14. iv. 20	14. iv. 20	14. iv. 20	14. iv. 20
No. of days after inoculation	21	21	21	21
Ratio of wilted to total leaves	0 : 10	6 : 12	8 : 12	0 : 12
Height of discoloured wood above stab	15 cm.	26 cm.	28 cm.	9 cm.
No. of days from inoculation to complete wilt	49	28	28	No wilt after 80 days

Table V.

		Tomato house		Cucumber house	
	Frame	Unshaded	Shaded	Unshaded	Shaded
Date of inoculation	14 iv. 20	14. iv. 20	14. iv. 20	14. iv. 20	14. iv. 20
No. of days from inoculation	21	21	21	21	21
Average temperature ° C.	17	22	20	26·3	25
Ratio of wilted to total leaves	6 : 10	3 : 10	1 : 10	0 : 10	0 : 10

While the results obtained are open to criticism because of the wide range of temperature to which the plants were submitted in any one position, certain empirical facts emerge which have been fully confirmed by observations in commercial nurseries. Chief among these is the beneficial effect which shade and temperatures above 24·0° C. have upon plants suffering from *Verticillium* wilt. Table IV shows that average temperatures of 16·6° C. and 20·0° C. are favourable to the rapid progress of the disease, that of 12·5° C. is unfavourable, while that of 25° C. practically inhibits it. It will be seen that the organism has travelled most rapidly up the stem, as indicated by the browning of the wood, at 16·6° C. and 20·0° C., and at these temperatures also complete wilt occurred most quickly. The results shown in Table V, while confirming the temperature relations, show the beneficial effect of shade. While plants in the unshaded tomato house readily wilted, those in the shaded house exhibited only slight wilting. Observations in commercial glass-houses have shown that temperatures between 15·6° C. and 24·0° C. with an optimum of 21·1 to 22·8° C. are favourable to the rapid progress of *Verticillium* wilt. Below 15·6° C. and above 24·0° C. this is exceedingly slow, while suitable shading partially counteracts the effect of low temperatures.

A series of experiments was next arranged, in which wilted plants were transferred to conditions of high temperatures to ascertain if they would recover and if such recovery would continue, when the plants were returned to lower temperatures. The results are tabulated below.

Table VI.

No. of wilted plants	Length of time in shaded cucumber house (aver. temp. 25° C.)	Effect of high temperature	Length of time after returning to an aver. temp. of 20·0° C. before plants again wilted
12	1 day	Recovery	15 hours
12	2 days	,,	15 ,,
12	7 ,,	,,	2 days
12	14 ,,	,,	3 ,,
12	30 ,,	,,	16 ,,
12	75 ,,	,,	30 ,,

Table VII.

No. of wilted plants	No. of days wilt has been visible prior to experiment	Percentage recovered in shaded cucumber house (aver. temp. 25° C.)	Percentage recovered in unshaded cucumber house (aver. temp. 25° C.)
20	2	100	100
20	7	100	100
20	14	100	100
20	21	100	90
20	30	100	80

The results in Table VI indicate that wilted plants recover when the average temperature is raised to 25° C. When such a temperature is operative for a short time, the effect is not a lasting one, for the plants rapidly wilt again when the temperature is lowered. Longer exposures to the higher temperature produce a more lasting result, for after 75 days at 25° C. the plants remained turgid for 30 days at a temperature favourable to wilt. In Table VII the percentage of wilted plants, which recover when transferred to a shaded house at an average temperature of 25° C., is compared with that of similar plants transferred to an unshaded house at the same temperature. Plants in different stages of wilt were used, from a series where the wilt was just commencing to a series in an advanced stage after 30 days' wilting. All the plants recovered in the shaded house, but only a portion in that which was not shaded. The plants which did not recover in the unshaded house, being the badly wilted ones, were probably desiccated before they had a chance to recover. These observations appear to justify the conclusion that temperature is a most important factor in controlling the Verticillium Wilt Disease of tomatoes, while shading is valuable because it assists the plant, probably by reducing transpiration. The minimum, optimum and maximum temperatures for growth in pure culture of the strains of *Verticillium albo-atrum* utilised for the inoculations were 4·4° C., 23·3° C. and 30° C. respectively, and it will be seen that the optimum temperature for infection coincides approximately with the optimum temperature for growth in pure culture. *Verticillium* wilt is distinctly a disease of moderately low temperatures and is therefore most severe in the spring and autumn.

Soil factors.

Experiments carried out with different soils show that there is no obligate relation between *Verticillium* wilt and any particular soil type. Generally speaking, however, plants on soils which contain a large amount of humus show more disease than those growing on soils of a poorer nature. Clay soils, in virtue of their greater water-holding capacity, are cooler than sandy soils, and plants grown upon them are more prone to wilt than those grown on the latter.

III. CONTROL.

Investigations to determine the chemical agents best suited to eliminate the disease organisms from the soil and also to ascertain the effect of different manurial treatments upon the incidence of the disease are in progress and will be reported upon later.

1. CULTURAL METHODS.

Cultural methods for controlling the wilt disease have been devised and tested with promising results in the Lea Valley. In places where the disease has been common in previous seasons it is advisable to grow a highly resistant variety such as Manx Marvel or Bide's Recruit. Care should be taken to protect the plants from any check in their development and to encourage slightly soft rather than hard growth. When *Verticillium* wilt appears, the temperature should be raised until the average day and night temperature is above 25° C. This may be done by suitably increasing the boiler heat, regulating the ventilation and closing the ventilators for two to four hours in the middle of the day. A light dressing of whitewash on the glass makes the conditions still more favourable for the plants. As little water as possible should be given to the roots as this aggravates the wilting, but a light overhead damping is beneficial. The development of fresh roots should be encouraged by mulching the base of the plant.

On one nursery 78 per cent. of the plants were showing symptoms of wilt disease before the above methods were enforced: a fortnight later only 10 per cent. remained wilted. In view of the fact that low spring temperatures favour infection by *Verticillium*, some advantage might be gained by planting later than is usually done, so that the higher summer temperatures may arrive before the plants are infected.

2. The elimination of sources of infection.

Edgerton (10) has pointed out that *F. lycopersici* develops much more rapidly in sterilised soil than in ordinary unsterilised soil. This has been found true for *V. albo-atrum*, for plants growing in imperfectly sterilised soil or re-inoculated sterilised soil yield a higher percentage of disease than those in unsterilised soil. This relation is accentuated if the soil be exceedingly rich in humus, as is the case with sterilised cucumber soil so frequently used for tomato propagation. The determination and elimination of infection thus becomes of vital importance. The fungal outgrowths at the base of dead diseased plants produce innumerable spores which become widely disseminated. These in themselves will not carry the fungus over the period of winter, but they readily germinate, and feeding on decaying plant material produce carbonised hyphae and microsclerotia, which are able to withstand winter conditions. Examination has been made of small pieces of plant remains, unearthed from nursery soils after the crop has been removed, and numerous tomato pathogens including *V. albo-atrum* have been found upon them. Thus it is important to remove completely all plants killed by wilt disease before cleaning up the nursery when the crop is finished and to remove carefully as much as possible of the general debris. The best way to remove the crop, when completed, is to sever each plant about 3 inches from the soil and remove all the aerial portions including leaves, etc., which have fallen to the ground, before attempting to remove the roots. If the surface is quite clean before the roots are removed, there is less chance of incorporating diseased material in the soil and the roots may then be carefully taken up, leaving behind only the very fine rootlets.

Another source of infection, which becomes more and more evident, is the contamination carried in "strikes" or baskets, and a considerable number of cases have been noted, where infection with various diseases has been traced to these articles. Baskets may be so mixed at the market that when they are returned, those from one nursery are sent to another, and so disease is spread. Baskets should not be taken near the growing plants for fear of introducing some new trouble, and during the winter months all baskets should be sterilised in readiness for the coming season. The importation of young plants from other nurseries is a procedure to be deprecated, for it is a fruitful means of disease dispersal. Contaminated water from surface wells is a constant source of infection with many diseases (1), and care should be taken to use a pure water supply. *V. albo-atrum* will infect a large number of cultivated plants, as well as certain trees, and while there is yet no direct evidence to show that the fungus may attack the common weeds around nurseries, it will probably be found that such is the case. It is desirable, therefore, that the immediate vicinity of nurseries be kept free from weeds, while potatoes and antirrhinums should not be permitted as they are susceptible to *Verticillium*.

In America, tomatoes resistant to the Fusarium Wilt Disease have been produced and an attempt is being made in this laboratory to raise a *Verticillium* resistant strain.

The author desires to express his gratitude to Dr W. B. Brierley of the Rothamsted Experimental Station for the many helpful suggestions and criticisms so kindly given during the course of this work, also to Mr W. Buddin, M.A., late assistant mycologist at this station for assistance in the preparation of the enzyme extracts.

SUMMARY.

1. "Sleepy Disease" or "Wilt" of tomatoes may be caused by one of two fungi, *Fusarium lycopersici* or *Verticillium albo-atrum.*

2. Massee's "diplocladium stage" of *F. lycopersici* has been shown to be *V. albo-atrum.*

3. The wilt producing fungi attack the roots and grow up through the vascular bundles into the stem, leaves and sometimes the fruits. The wood of a diseased plant is a light or dark brown colour.

4. The average temperature is a "limiting factor" in determining which fungus is active. *F. lycopersici* grows best at an average temperature of 27·8–28·9° C. If the temperature remains constantly much below this, little infection results. *V. albo-atrum* develops well at temperatures

from 15·6–24·0° C., being most active at 21·1–22·8° C. Above an average temperature of 25° C. little infection occurs.

5. The average temperature conditions existing in glasshouses in this country are generally too low for *F. lycopersici* and consequently it is rarely found as a cause of tomato wilt. The relatively low temperatures are favourable to *V. albo-atrum*, which accordingly is the most important cause of wilt.

6. Wilted plants soon die under conditions of low temperature, but if the average temperature be raised above 25° C., they recover and will bear a crop so long as the high temperature is maintained. When the temperature again drops, wilt reappears and death results.

7. *V. albo-atrum* from tomato readily induces wilt in the potato, egg-plant, snapdragon, cotton, pepper plant and cucumber, and produces a stunted condition of the sycamore and elm.

8. A number of different strains of *V. albo-atrum* have been isolated which vary in their rate of growth, the amount and rate of production of microsclerotia, and in colour production; but no evidence has been obtained to show that there may be different strains restricted to different varieties of tomatoes.

9. In pure culture the fungus has been shown to produce a large number of enzymes and there are strong indications that substances of a toxic nature play an important part in producing wilt.

10. There is a distinct relation between hardness of growth and susceptibility to wilt; the harder growing varieties and plants suffering from starvation or a severe check in the young stages being most susceptible to attack. Most varieties of tomatoes cultivated in this country are susceptible to *Verticillium*, but Manx Marvel has proved to be practically immune and Bides' Recruit highly resistant.

11. Certain cultural devices, including regulation of the temperature and shade, have been devised which assist "wilted plants" to recover.

12. Further investigations upon soil sterilisation and the production of resistant varieties are in progress.

APPENDIX.

Since this paper was written, a wilt disease of the sweet-pea has occurred in certain commercial nurseries where this crop is grown in the early part of the year before a tomato crop. The young seedlings showed first symptoms when about 6 inches high, the lower leaves turning yellow and withering. The desiccation advanced rapidly to the top of the seedlings, which then died. *Verticillium albo-atrum* was isolated and inoculation results proved it to be the cause of the disease. A series of cross-inoculations were undertaken with *V. albo-atrum* from the tomato, cucumber and sweet-pea, upon tomatoes, cucumbers and sweet-peas. In every case a definite wilt was produced and examination of the various isolations indicated that the fungi from these host plants are identical.

BIBLIOGRAPHY.

(1) BEWLEY, W. F. and BUDDIN, W. (1921). *Ann. App. Biol.* VIII, No. 1.
(2) BRANDES, E. W. (1919). *Phytopath.* IX, No. 9.
(3) BROWN, W. (1915). *Ann. Bot.* XXIX, No. 115.
(4) CARPENTER, C. W. (1918). *Jour. Agr. Res.* XII, No. 9.
(5) CLAYTON, E. E. (1920). *Phytopath.* X, No. 1.
(6) CRABILL, C. H. and REED, H. S. (1915). *Biochem. Bull.* IV, No. 13.
(7) DIXON, H. H. (1914). *Proc. Roy. Dublin Soc.* XIV (N.S.).
(8) —— (1914). *Transpiration and the ascent of sap in plants.* Macmillan's Science Monographs.
(9) EDSON, H. A. and SHAPOVALOV, M. (1920). *Jour. Agr. Res.* XVIII, No. 10.
(10) EDGERTON, C. W. (1918). *Phytopath.* VIII, No. 1.
(11) HASKELL, R. J. (1919). *Phytopath.* IX, No. 6.
(12) KLEBAHN, H. (1913). *Mycol. Centr.* III, Heft 2.
(13) LAURITZEN, J. I. (1919). *Phytopath.* IX, No. 1.
(14) MASSEE, G. (1896). *Jour. Roy. Hort. Soc.* XIX.
(15) —— (1910). "Diseases of cultivated plants and trees."
(16) PETHYBRIDGE, G. H. (1916). *Sci. Proc. Roy. Dublin Soc.* XV (N.S.), No. 7.
(17) REINKE, J. and BERTHOLD, G. (1879). *Untersuch. Bot. Lab. Univ. Gottingen,* Heft 1.
(18) TISDALE, W. B. (1917). *Phytopath.* VII, No. 7.
(19) VAN DER LEK, H. A. A. (1918). *Over. u. d. Meded. v. d. Land.* D. XV.
(20) WOLLENWEBER, H. W. (1913). *Ber. Deut. Bot. Gesell.* XXXI, Heft. 1.
(21) —— (1913). *Phytopath.* III, No. 1.

EXPLANATION OF PLATES IV–VII

PLATE IV.

Fig. 1. Diseased tomato stem cut longitudinally to show the browned wood caused by *V. albo-atrum.*

Fig. 2. Old diseased tomato stem showing the fungal outgrowth at the base.

Fig. 3. Micro-photograph of *V. albo-atrum.*

PLATE V.

Fig. 1. (1) Wilted plant six weeks after inoculation with *V. albo-atrum.*
(2) Control plant.

Fig. 2. This photograph shows the wilted plant in Fig. 1 after being submitted to shade and an average temperature of 25° C. for 30 days. The wilted leaves have fallen off, but the plant has recovered and made good growth in the top.

Fig. 3. (1) Plant inoculated with *V. albo-atrum* at an average temperature of 20·0° C. (2) Control plant.

Fig. 4. (1) Recovery which resulted from placing the wilted plant (Fig. 3 (1)) under an average temperature of 25° C. for 24 hours. (3) Inoculated plant left at 20·0° C. showing wilt.

PLATE VI.

Fig. 1. Antirrhinum. (1) Control. (2) Inoculated with *V. albo-atrum* by a prick in the stem: wilt shows on one side only. (3) Plant grown in infected soil.

Fig. 2. Potato. (1) Control. (2) and (3) Inoculated with *V. albo-atrum.*

PLATE VII.

Fig. 1. Cotton plant. (1) Control. (2) Inoculated with *V. albo-atrum.*

Fig. 2. Sycamore. (1) Control. (2) Inoculated with *V. albo-atrum.*

(*Received Oct.* 18*th*, 1921)

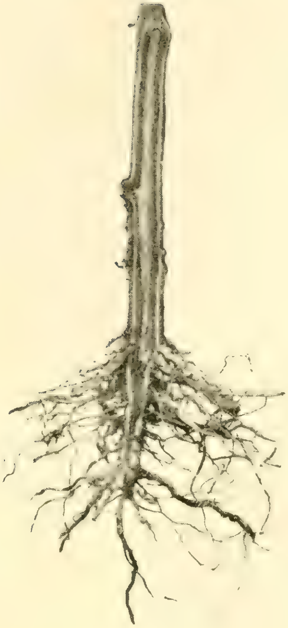


Fig. 1.

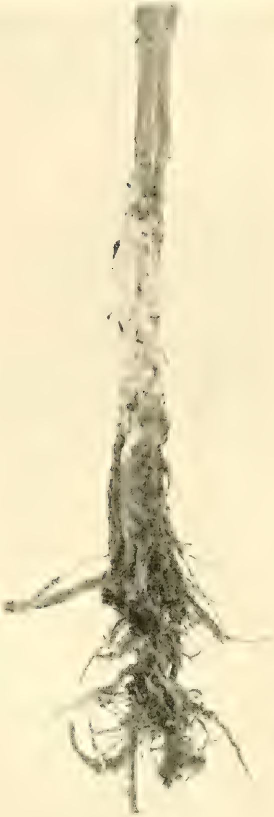


Fig. 2.

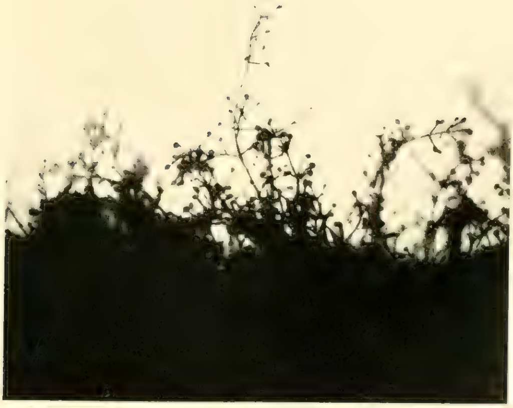


Fig. 3.

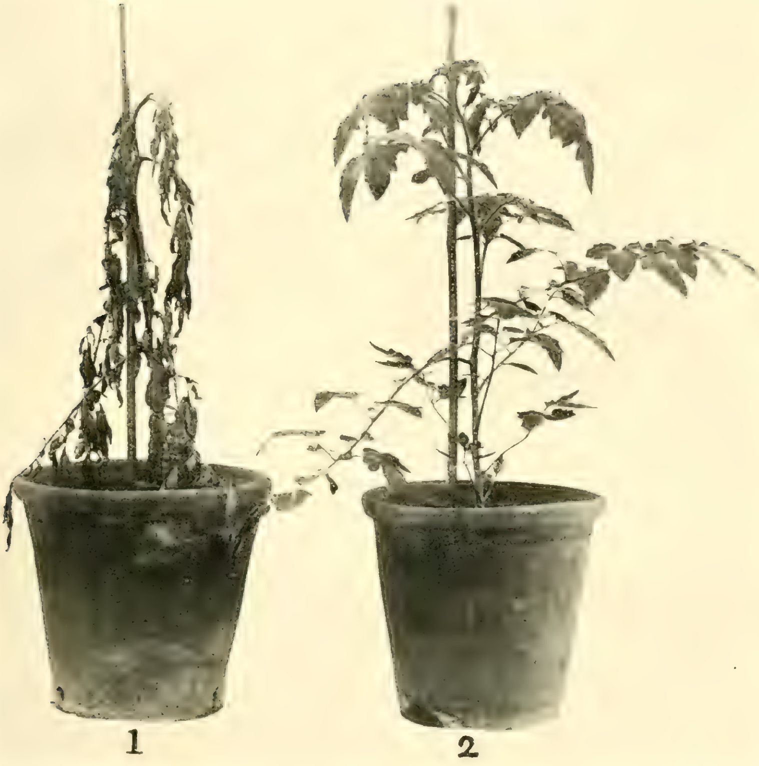


Fig. 1.

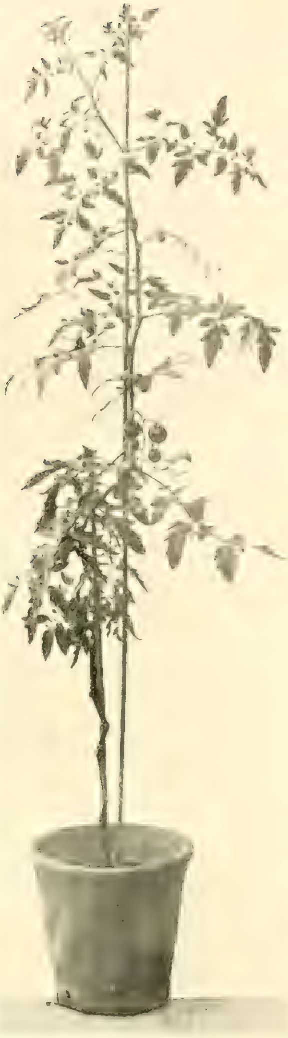
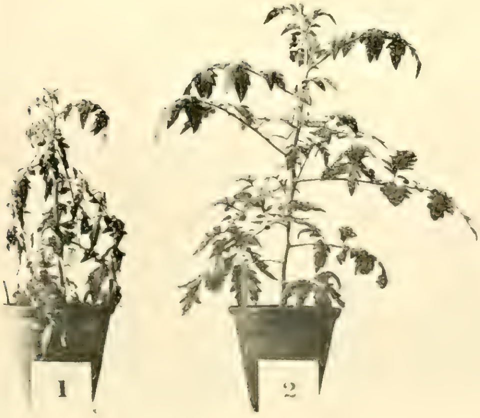
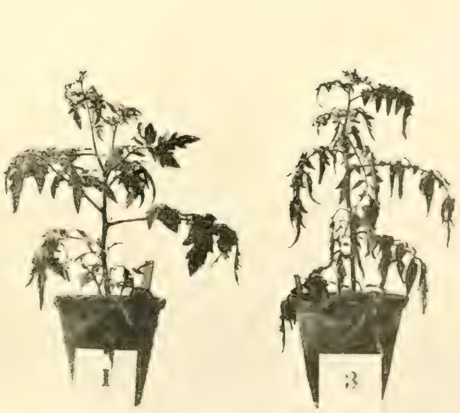


Fig. 2.





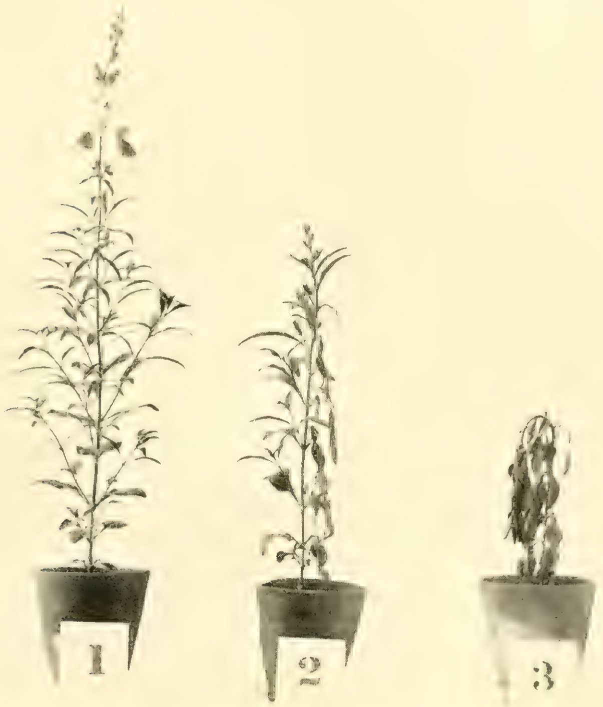


Fig. 1.

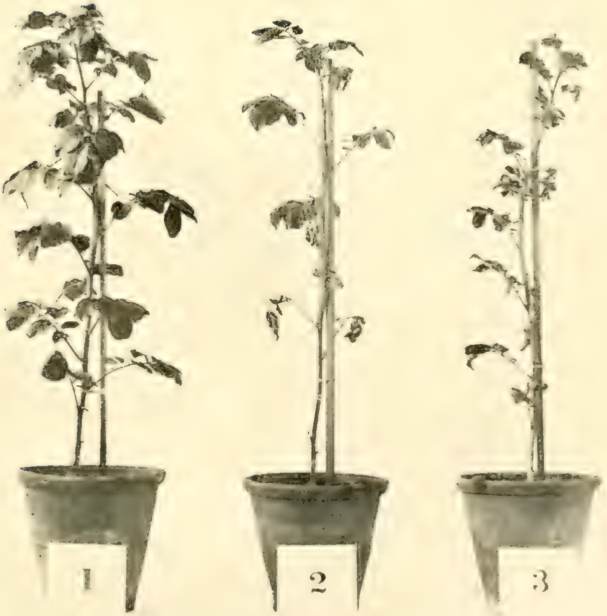


Fig. 2.

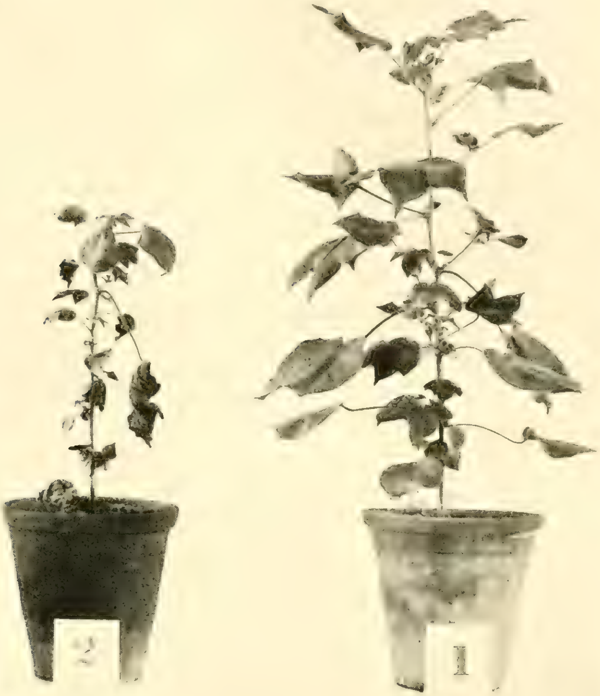


Fig. 1.

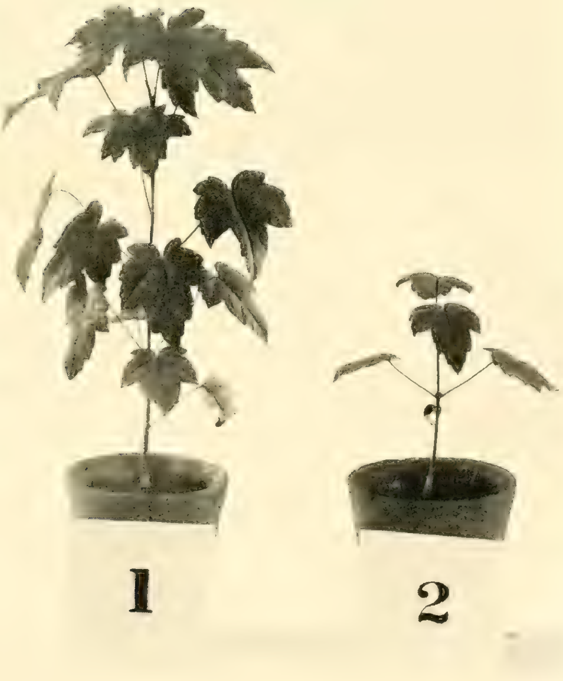


Fig. 2.

BIOLOGICAL STUDIES OF *APHIS RUMICIS* LINN. REPRODUCTION ON VARIETIES OF *VICIA FABA*.

By J. DAVIDSON, D.Sc.

(From the Entomological Department, Institute of Plant Pathology, Rothamsted Experimental Station, Harpenden.)

WITH A STATISTICAL APPENDIX BY

R. A. FISHER, M.A.

(With 1 Text-figure.)

I. INTRODUCTION.

The following paper is a continuation of previous work (1) and gives results showing the varying reproductive capacity of *Aphis rumicis* on eighteen different varieties of field beans.

My sincere thanks are due to the following gentlemen who very kindly supplied me with seed for these experiments: Prof. J. Percival, M.A., University College, Reading; Mr R. M. Wilson, Principal, East Anglian Institute of Agriculture, Chelmsford; and The Director, Sveriges, Utsädesförening, Svalöf, Sweden.

My thanks are also due to Mr R. A. Fisher for his kindness and assistance with the statistical considerations involved in this paper.

The following abbreviations are used in the text: w. v. ♀ = winged viviparous female; a. v. ♀ = apterous viviparous female; 1st v. gen. = 1st, 2nd, etc. viviparous generations.

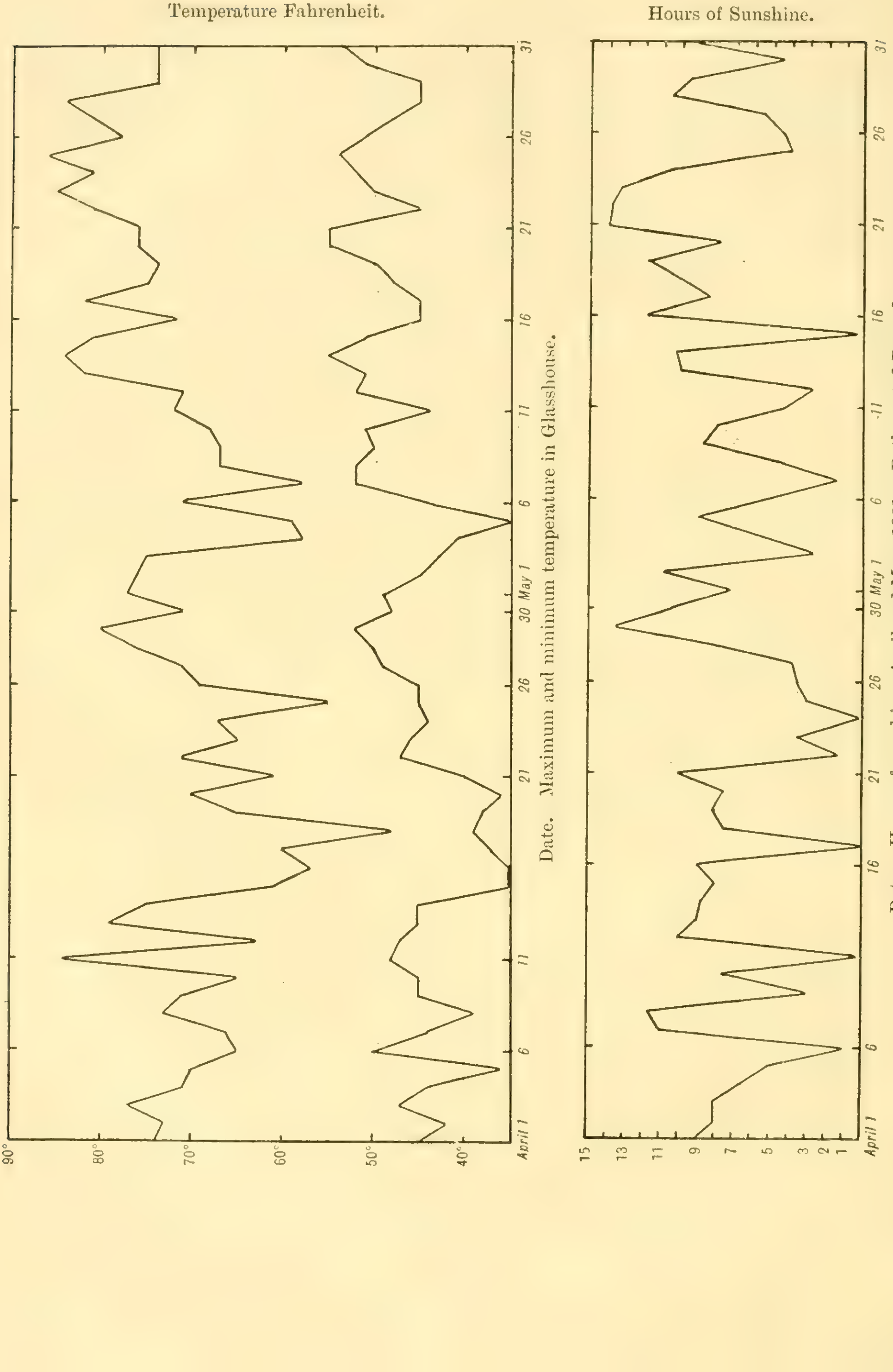
II. METHODS EMPLOYED.

The plants were grown in unmanured soil to which 10 per cent. of sand was added to prevent "caking" of the surface. Ordinary flower pots, 10 inches in diameter, were used. These were coated on the outside with a layer of wax in order to prevent excessive evaporation. The plants were kept covered with muslin bags throughout the experiments[1].

All the experiments were carried out under similar conditions in a large glasshouse specially designed with large windows and doors. The

---

[1] The amount of light cut out by these covers was estimated with a thermopile and found to be about 22 per cent. There was very little effect on the growth of the plant.



Date. Maximum and minimum temperature in Glasshouse.

Date. Hours of sunshine, April and May, 1921. Rothamsted Records.

Fig. 1.

maximum and minimum daily temperature in the glasshouse throughout the experiments is shown in Fig. 1.

There were six pots of each variety of field beans and six pots of garden Prolific Longpod broad beans. In all cases each pot contained a single plant. The latter variety of beans, which gave a high figure of infestation in the 1920 experiments, is taken as the standard to which the other varieties are referred in order to get relative figures of infestation. One plant of each variety was the "stock" plant for the variety, and the remaining five plants were utilized for the infestation tests.

The seeds were all sown on 25. iii. 21.

The aphids used in the experiments (*Aphis rumicis*) were derived from one egg, all being the offspring of one Fundatrix. Oviparous females, which developed in the colonies in the 1920 experiments, laid eggs in October 1920 on *Euonymus europaeus*. Some of the eggs commenced to hatch out on 8. iii. 21, and one Fundatrix was isolated on *Euonymus europaeus* on 25. iii. 21. This individual was the "stem-mother" of all the individuals used in the present experiments. As the individuals in each generation became adult they were isolated. The Fundatrix produced a. v. ♀♀ of 1st v. gen. These gave rise to a mixed progeny of w. v. ♀♀ and a. v. ♀♀ in the 2nd v. gen. Two winged migrants were transferred on 26. iv. 21 to each of the "stock" plants referred to above. On these they produced a. v. ♀♀ of 3rd v. gen. When these a. v. ♀♀ were almost adult and before they actually began to reproduce, the five plants of each variety were separately infected on 11. v. 21 with an a. v. ♀ derived from the stock plant of the variety concerned. The date and time when the a. v. ♀ on each plant began to reproduce was recorded (*vide* Tables) and reproduction was then allowed to go on for 14 complete days. The total number of aphids produced on each plant at the end of that period was then counted.

It will be noted that the infections of the different plants were made on the same day and that the 14-day reproduction period extended over practically the same period for all the varieties, thus ensuring that factors of temperature, humidity and sunshine were the same for all.

Further points which should be considered in experiments of this kind have already been given in the paper referred to above.

III. DISCUSSION.

Tables I and II shows the results obtained for the 18 varieties of field beans. These may be compared with the results obtained for Prolific Longpod (XIX) which is taken as the standard in order to fix the relative values of susceptibility of the other varieties.

Table I.

Variety	No. of variety	No. of plant	Date in May		Total aphids produced	Mean figure of infestation and probable error
			a. v. ♀ commenced producing	aphids killed off		
Bohus beans (Sweden)	I	1	13	27	1275	1018±51·3
		2	,,	,,	1058	
		3	,,	,,	967	
		4	,,	,,	904	
		5	,,	,,	884	
		6		Stock plant		
Mazagan	II	7	12	26	1188	1007±51·1
		8	,,	,,	1154	
		9	,,	,,	1068	
		10	,,	,,	992	
		11	,,	,,	632	
		12		Stock plant		
English field	III	13	13	27	978	737±43·7
		14	,,	,,	759	
		15	,,	,,	680	
		16	,,	,,	636	
		17	,,	,,	631	
		18		Stock plant		
Heligoland	IV	19	14	28	738	616±39·9
		20	16	29	723	
		21	14	28	622	
		22	,,	,,	502	
		23	,,	,,	497	
		24		Stock plant		
Scotch var. Granton	V	25	13	27	753	591±39·1
		26	,,	,,	677	
		27	,,	,,	544	
		28	,,	,,	542	
		29	,,	,,	441	
		30		Stock plant		
Winter beans (Dorset)	VI	31	13	27	641	564±38·2
		32	,,	,,	630	
		33	,,	,,	575	
		34	12	26	542	
		35	13	27	430	
		36		Stock plant		
Winter beans (Somerset)	VII	37	13	27	656	513±36·4
		38	,,	,,	525	
		39	,,	,,	501	
		40	,,	,,	458	
		41	12	26	426	
		42		Stock plant		
Winter beans (Essex)	VIII	43	13	27	541	451±38·2
		44	,,	,,	536	
		45	,,	,,	450	
		46	,,	,,	276	
		47	,,	,,	—	
		48		Stock plant		
Spring tick (Suffolk)	IX	49	13	27	487	419±32·9
		50	,,	,,	413	
		51	,,	,,	407	
		52	,,	,,	396	
		53	,,	,,	391	
		54		Stock plant		
Carse beans	X	55	13	27	650	415±32·8
		56	,,	,,	477	
		57	,,	,,	425	
		58	14	28	349	
		59	13	27	176	
		60		Stock plant		

Table II.

Variety	No. of variety	No. of plant	Date in May		Total aphids produced	Mean figure of infestation and probable error
			a. v. ♀ commenced producing	aphids killed off		
Kilbride beans	XI	61	13	27	522	423 ± 33·1
		62	,,	,,	446	
		63	,,	,,	441	
		64	,,	,,	391	
		65	,,	,,	317	
		66		Stock plant		
Winter beans (Hertfordshire)	XII	67	13	27	593	404 ± 32·4
		68	,,	,,	461	
		69	,,	,,	384	
		70	,,	,,	320	
		71	,,	,,	263	
		72		Stock plant		
Small tick	XIII	73	13	27	648	410 ± 32·6
		74	,,	,,	383	
		75	,,	,,	374	
		76	,,	,,	335	
		77	,,	,,	312	
		78		Stock plant		
Winter beans (Berkshire)	XIV	79	13	27	468	371 ± 34·7
		80	,,	,,	406	
		81	,,	,,	388	
		82	,,	,,	222	
		83	,,	,,	—	
		84		Stock plant		
Winter field (Cheshire)	XV	85	13	27	457	375 ± 31·2
		86	,,	,,	413	
		87	,,	,,	412	
		88	,,	,,	332	
		89	,,	,,	263	
		90		Stock plant		
Scotch spring beans	XVI	91	13	27	495	391 ± 31·8
		92	,,	,,	465	
		93	,,	,,	363	
		94	,,	,,	333	
		95	,,	,,	299	
		96		Stock plant		
Winter bean (Suffolk)	XVII	97	14	28	433	286 ± 27·2
		98	,,	,,	333	
		99	,,	,,	326	
		100	,,	,,	184	
		101	,,	,,	156	
		102		Stock plant		
Vicia narbonensis	XVIII	103	14	28	129	37
		104	15	29	33	
		105	,,	,,	9	
		106	14	28	11	
		107	15	29	5	
		108		Stock plant		
Prolific Long-pod	XIX	109	13	27	1205	1037 ± 51·8
		110	,,	,,	1147	
		111	,,	,,	1015	
		112	,,	,,	969	
		113	,,	,,	848	
		114		Stock plant		

The regular grouping of the figures showing the total number of aphids produced in 14 days (column 6) indicates that there is a significant difference in the degree of infestation of different varieties over a given period of time.

If we take the arithmetic mean of the total numbers produced on the five plants in each variety (column 7) we obtain for each variety a mean figure of infestation resulting from one apterous mother in a 14-day period. To this is attached its probable error calculated as explained in the Appendix.

The varieties therefore may be grouped into six distinctive classes, each class having a mean figure of infestation as shown in Table III. The mean figure of infestation for each class is taken as the arithmetic mean of all the means of the varieties included in the class.

Table III.

Class and mean figure of infestation	Prolific Longpod 1037	A 1012	B 737	C 570 ± 19·2	D 407 ± 11·1		E 286	F 37
Varieties	XIX	I	III	IV	VIII	XII	XVII	XVIII
		II		V	IX	XIII		
				VI	X	XIV		
				VII	XI	XV		
					XVI			
Degree of susceptibility	Standard variety taken as 100 %	98 %	71 %	55 %	39 %		27 %	3 %

Referring each class to the variety Prolific Longpod (XIX), the mean infestation figure for which is 1037, the relative degree of susceptibility of the varieties in each class may be expressed in percentages as shown in Table III.

Conversely by subtracting these percentages from 100, one gets the relative degree of resistance to infestation of the varieties in the different classes.

After 14 days reproduction on varieties in class A, the plants may be considered as fairly heavily infested, while the varieties in class D have only a moderate infestation and those in class E less so. Class F has almost a negligible infestation.

In 21 days the varieties in class A would be practically destroyed by the aphid infestation, those in classes D and E less so and in class F almost negligible.

There is no class here representing complete immunity from attack but the aphids were compelled to stay on these plants. In nature winged migrants would select their host and probably not reproduce on unfavourable varieties.

The classes evidently show significant differences in the degree of infestation in a given period.

It would appear probable that owing to the influence of the cell sap of the varieties concerned on the aphid metabolism, the rate of reproduction is considerably affected and thus under the same conditions of environment, the chances of infestation occurring are greater with some varieties than others.

This in itself is an important economic consideration in that, with the prospects of heavy rain or broken weather conditions (unsuitable for aphids) within a two or three weeks' period of fine weather, the chances of varieties in classes D, E and F recovering from aphis attack, are much greater than would be the case with varieties in class A.

Plant-breeding experiments would show whether susceptibility or resistance is a specific mendelian character. It seems feasible to consider tentatively, that the factor or factors (genes) which make for high resistance as in *Vicia narbonensis*—which as discussed below probably represents the prototype of *Vicia faba*—may have been present in the original wild bean, and that this character has been lost or modified in the process of selection in the cultivated varieties.

A theoretical discussion of this question of varying susceptibility would hardly be profitable at this stage. It would appear, however, that the factor or factors concerned are associated with the general physiology of the plant and have an influence on the cell sap.

If one factor only is concerned, it should not be a difficult matter to trace it by cross breeding experiments. On the other hand, if the character of resistance is due to an interworking of a number of factors, the problem becomes an extremely complex one.

It is interesting to consider variety XVIII (*Vicia narbonensis*) in this respect.

Some authorities consider this species as a prototype of the cultivated *Vicia faba*.

According to De Candolle (2) the bean has been cultivated from prehistoric times, and may have been distributed during the early migration of man. Some thousands of years ago it was probably established wild in two areas, namely, south of the Caspian sea and North Africa.

Vicia narbonensis most nearly represents the wild prototpye of the modern cultivated race. It is found wild to-day in the Mediterranean area; east towards the Caucasus; in Northern Persia and Mesopotamia[1].

---

[1] I have tried Prolific Longpod ♂ × *Vicia narbonensis* ♀ in 1920 and have reason to believe that three pods which came to maturity are successful crosses. The seeds from these pods are being carried to the F_2 generation.

The improved conditions associated with good cultivation, manurial treatment, etc., may to some extent influence the degree of susceptibility to aphis attacks. In the case of wild and cultivated varieties, the grouping of the 18 varieties used in these experiments (Table III) indicates that these factors are not the only considerations. The infestation figures obtained for the 10 varieties of broad beans experimented with in 1920 (*loc. cit.*) showed no significant difference in the degree of susceptibility.

IV. SUMMARY.

The reproduction of *Aphis rumicis* was tested on 18 varieties of field beans and the results compared with the reproduction on Prolific Longpod broad beans.

The aphids used in the experiments were the offspring from one Fundatrix.

The experiments were all carried out under similar conditions. Five plants of each variety were tested, and the total number of aphids produced on each plant, from one a. v. ♀, in 14 days was counted.

The mean values of infestation for the varieties range from 37 to 1037, *vide* Tables I and II.

These mean values allow of the varieties being grouped into classes representing various grades of susceptibility, ranging from 98 per cent. to 3 per cent. *vide* Table III.

Vicia narbonensis has a very low susceptibility. The results obtained indicate that resistance or susceptibility may be largely determined by genetic factors in the plant.

REFERENCES.

(1) Davidson, J. (1921). *Ann. Appl. Biol.* VIII, 51–65.
(2) De Candolle, A. (1884). *Origin of Cultivated Plants*, 316–321.

APPENDIX

STATISTICAL CONSIDERATIONS INVOLVED IN TABLES I AND II OF THE ABOVE PAPER.

By R. A. FISHER, M.A.

Fellow of Gonville and Caius College; Statistician, Rothamsted Experimental Station, Harpenden.

The discussion of the probable error to be attached to Dr Davidson's aphis infestation numbers, involves points of statistical interest, which have hitherto not, in print at least, received adequate treatment.

Since only five infections were made on each of the varieties tested it is clear that only the roughest estimate of the standard deviation can be based upon the data for a single variety. For a standard deviation estimated from a sample of five is not only subject to very large errors of sampling, but is distributed in a markedly skew manner; on the other hand, the causes of variation, whatever they may be, must be closely analogous in all the varieties tested; and this fact should enable us to make use of the information supplied by the whole of the material, to estimate with some accuracy the probable error to be ascribed to each value.

The process of obtaining a single probable error from the deviations of a number of distinct groups has been applied successfully in cases where it may be assumed that the groups are equally variable; as is the case when a correlation ratio is determined on the assumption of the equal variability of the arrays. In the case of the infestation numbers no such assumption is *a priori* plausible, for setting aside the lowest mean which is evidently exceptional, the 18 means range from 286 to 1037. Moreover, an inspection of the figures for the individual plants shows that the higher numbers are, as is to be expected, actually the more variable. There exists, however, a class of distribution, of which the Poisson Series is the classical example, in which the variance is proportional to the mean.

To test whether this is the case with the infestation numbers, the quantities

$$\frac{n\mu_2}{\bar{x}}$$

were calculated for each variety, where $\bar{x}$ is the mean and μ_2 the second moment of each sample; $n = 5$ in every case but two, for which only four counts were available. In these cases the quantity was increased by one-third, and used with the others to obtain the aggregate. Adding the 18 quantities obtained from the several varieties, and dividing by $18\,(n-1)$, we find that on the average the variance is nearly 28·5 times the mean. If now the variance is proportional to the mean we have for each variety

$$\sigma^2 = 28{\cdot}5\bar{x}$$

and the probable error is thence calculated with 18 times the weight with which it would be calculated from a single variety.

A precise test of the accuracy of the above assumption is afforded by the distribution of

$$\frac{n\mu_2}{\sigma^2}$$

for, if the standard deviations have been correctly calculated, this must be distributed as in χ^2 when $n' = 5$ in Elderton's table of Goodness of Fit. For the 17 values we have the following comparison.

	$\overbrace{\text{Expected} \quad \text{Observed}}^{\frac{nn_2}{\bar{x}}}$			
	Expected m	Observed	e	$\frac{\epsilon^2}{m}$
0–2	4·49	4	$-$ ·49	·05
2–4	5·61	8	$+$2·39	1·02
4–6	3·52	1	$-$2·35	1·03
6–8	1·83	2		
8–10	·87	1	$+$ ·44	·12
10–	·69	1		
				$x^2 = 2{\cdot}22$

Since we have introduced one empirical value we must take $n' = 3$, and obtain $P = {\cdot}336$. Thus in 33 cases out of 100 we should expect a worse fit to occur by chance, and this shows that the totality of the observations shows no significant deviation from the set of standard deviations which we have assigned to them.

With a knowledge of the probable errors of the infestation numbers it is possible to test to what extent the several varieties may be grouped together as possibly identical in respect of aphid infestation. From the genetic standpoint it is of the highest importance to determine the continuity or discontinuity of susceptibility; and it is only too frequently that statisticians infer the continuity of a variable quantity without testing to what extent the apparent continuity is due to genuine continuity, due to a multitude of underlying genetic factors, and to what extent a real discontinuity has been obscured by chance variation. In the present case the homogeneity of certain groups is suggested by the mean values. Without being able to test this delicate point with rigour, it is worth while to note that the numbers obtained from certain groups are consistent with the supposition of identical susceptibility.

The group of three varieties (Nos. I, II, XIX) giving mean infestation numbers 1007 to 1037, may be regarded as samples from a single group with mean infestation number at 1020. The standard deviation of the individual plants from this value is 167, and that calculated as explained above is 171.

The variety No. III with infestation number 737 appears to be isolated.

The four varieties (Nos. IV, V, VI, VII) with infestation values 513 to 616 are distributed consistently with the supposition that they are samples of a group with mean value at 570, the observed standard deviation is 105 while the calculated one is 127.

The nine varieties (Nos. VIII, IX, X, XI, XII, XIII, XIV, XV, XVI) with infestation numbers 370 to 451 are distributed about a mean of 407, the observed standard deviation is 103 as against a calculated value 108. The two remaining varieties (Nos. XVII and XVIII) do not seem to be assignable to any other group, and may be regarded at least provisionally as each representing a separate grade of immunity; the very low susceptibility and the high variability of the last variety (No. XVIII) especially makes it worth a more extensive study.

(*Received Oct. 25th*, 1921.)

THE TOXIC ACTION OF TRACES OF COAL GAS UPON PLANTS

By J. H. PRIESTLEY.

The fact that under certain conditions small quantities of unburnt coal gas may produce very deleterious effects upon vegetation is obviously of considerable economic importance. Both in Germany and in the United States these effects have frequently been under investigation but in this country they have attracted less attention. The earlier recognition of this type of poisoning of vegetation in other countries may be due in part to the general differences in composition between the illuminating gases employed, but the composition of coal gas in this country varies within wide limits and a change in the general methods of coal carbonisation might at any time so alter the average composition of British coal gas that this phenomenon might become of greater importance in British horticulture. Gas poisoning of vegetation probably occurs at present in this country but, as the American workers have found (5, *loc. cit.* p. 28), the absence of specific diagnostic characters enabling the damage to be accurately assessed, makes it difficult to appraise the economic importance of this particular source of injury to plants.

As the result of experiments in another field of investigation, the writer's attention was drawn to some of the cases of injury produced experimentally upon plants by the use of coal gas, and the structural changes resulting from gas poisoning were therefore examined. The preliminary results of this examination appear so significant that they are presented in this paper.

As so little attention has been devoted to the subject in this country, a brief summary is first given of the recorded effects of gas poisoning and of the definite information obtained by both German and American workers as to the constituents in the gas responsible for the toxic action. Very varied phenomena have been discussed in connection with the subject and throughout this paper attention will be restricted to cases where injury is reported as the result of the action of relatively low concentrations of coal gas. In such cases a common cause for the toxic effect produced seems to be clearly demonstrated. On the other hand when coal gas in high concentration displaces the air normally present around and within the plant, secondary effects may well be produced, such as asphyxiation, as found by Kosaroff(7) in his experiments with hydrogen and carbon dioxide.

The Nature of the Injuries produced by Traces of Coal Gas.

In the normal flowering plant investigators are agreed that the organ most sensitive to the toxic action of traces of the gas, is the root (5, 1, 3). The normal leafy stem appears to be very little affected. Knight and Crocker(6) have drawn attention to the extraordinary sensitiveness of etiolated pea epicotyls, whilst Harvey(4) has shown that the growing leaves of Ricinus are exceedingly sensitive and Crocker and Knight(2) report similar behaviour with the flower buds of carnation. The reactions of these sensitive plant structures occur at astonishingly low concentrations of the gas and thus support the earlier observations of German workers on the deleterious effects produced by laboratory air contaminated with small quantities of unburnt gas, upon the growth of etiolated seedlings of the pea, vetch, or lentil (9, 15) and upon the etiolated shoots of the potato (16).

As the result of a great deal of work, mainly by the investigators quoted above, there is general agreement both as to the structural effects produced upon the plant and as to the constituents of the gas responsible for the effect. There is also agreement that the actively growing organs of the plant are far more sensitive than resting structures.

In the case of roots, tubercles near the tip of the growing root are reported by Doubt(3) and Harvey and Rose(5), a lessened growth in length together with local increase in girth is reported by Molisch(8), Richards and McDougal(14), whilst both Molisch(8) and Harvey and Rose(5) report irregular curvatures in the root. Similarly in the case of the etiolated stem, Neljubow(9) reported and Knight and Crocker(6) confirm, a series of reactions on the part of the plants which develop progressively with rising concentrations of the gas. These reactions in order are (1) reduction in rate of growth, seedling remaining erect, (2) diageotropic curvature with slight swelling of stem, (3) an increased swelling with further reduction of growth, (4) complete cessation of growth in length with much swollen stem curved diageotropically, (5) death of seedling. Richter(15) has studied the structure of the swollen zone of the stem and notes an abundant development of collenchyma together with numerous cracks in the surface which are lined with cork.

In the carnation the flower bud fails to open, the petals are discoloured and withered, leaving the stigmas protruding. In the leaves of Ricinus and in other leafy shoots, epinastic movements are the first indications of injury. Harvey's experiments upon Ricinus show that in this plant leaf-fall occurs with relatively very low concentrations of the gas around the leafy shoot. In other experiments where leaf discoloration or leaf-fall has been reported it is in all probability a secondary effect following upon damage done to the root system by the gas.

Many statements draw attention to the effect of the gas upon superficial corky tissue; Stone (17) reported proliferation of tissue at the lenticels of willow slips growing in water charged with the gas, Doubt (3) reports the development of soft spongy tissue in the lenticels of Hibiscus and Sambucus, and upon the leaf scars of Lycopersicum, also the appearance of deep longitudinal cracks in the bark of many woody plants, apple, pear, ash, etc. Harvey and Rose (5) also noticed proliferation at the lenticels of roots when gas was slowly passed through the soil, whilst Richter (15) had noted the same effect produced by tobacco smoke.

Toxic Constituents of the Gas.

Nelbujow (9) was the first to show experimentally that the effect upon etiolated seedlings might be traced to unsaturated hydrocarbons which could be removed by passing the gas over red hot copper oxide. He also showed that ethylene in particular, if present alone at a very high dilution indeed, was capable of producing similar effects upon plants. The exhaustive experiments of Crocker and his colleagues (2, 3, 4, 5, 6) have placed this result beyond all doubt, and they show also that other possible poisonous constituents such as prussic acid, carbon monoxide, sulphur dioxide, etc. if present in the atmosphere in concentrations equivalent to that provided by the toxic amount of illuminating gas or tobacco smoke, are completely without action upon the plant. Wehmer (18, 19, 20), who has recently attributed the toxic action of coal gas, first of all to benzol and its homologues and sulphur compounds, and then later (21, 22) to hydrocyanic acid gas, appears to have been unaware of the earlier work of Crocker and his colleagues. In any case his experiments are not strictly comparable as he works with very high concentration of coal gas, in many experiments 100 per cent.; furthermore, he does not obtain toxic effects when these constituents are introduced into air at concentrations equivalent to those in which they occur in toxic concentrations of coal gas. The proportion of unsaturated hydrocarbons present in the illuminating gas in Germany is frequently very high, whilst in America (where water gas is the illuminant) it is also usually higher than in this country; this probably provides the reason for the phenomena being first on record in Germany and then found of practical importance in America, whilst they are nearly ignored in this country.

When ethylene is used alone in air the experiments of Knight and Crocker (6) show that a concentration of one part in ten million will retard the growth of the etiolated epicotyl of the pea, whilst a concentration of four parts in ten million will produce the full triple response, retardation of growth, increase of girth and the diageotropic position. Even with the illuminating gas at Leeds, which contains a relatively small concentration of ethylene (about 2 per cent.), it is very easy to obtain the full effect when growing etiolated seedlings in a laboratory where gas is frequently used, and experimentally it can be induced with the utmost ease. It was the consideration of the experiments of Knight and Crocker upon the etiolated pea seedling that led the writer to the experimental work which may go far to explain the mechanism of the effect produced.

The Mechanism of the Toxic Action of the Gas.

The significance of the structural effects produced by ethylene upon the etiolated epicotyl of the pea was immediately evident to the writer in the light of some observations recently made on etiolated plants. This work is now being published (11), but it is necessary to state here some results obtained as to the structure of the etiolated epicotyl of the pea and as to the function of an endodermis. In conjunction with Dr J. Ewing (11 *f*) the writer has found that in many plants grown under etiolation conditions, the stem contains a well marked functional primary endodermis from the base of the stem to just behind the growing apex. In the stem of the same plant grown in the light such an endodermis is only present for a very short distance above the ground level. It will be shown elsewhere that in these plants the special structural and morphological features characteristic of etiolation, are largely the result of the presence of this endodermis.

By a primary endodermis is meant an unbroken cylinder of cells in which the Casparian strip forms a continuous network in the substance of both transverse and longitudinal radial walls. An examination of the literature shows that the presence of this primary endodermis in stems grown in the dark has occasionally been noted, but its widespread occurrence under these conditions and the significance of its presence have been completely neglected. This may be partly due to the fact that the presence of the strip may easily be missed unless the sections are treated with a special view to its demonstration. Many methods will be found excellent for this purpose; sections cleared by boiling in potash or in Eau de Javelle, washed, and then stained in phloroglucin and hydrochloric acid, or in gentian violet or safranin, will show the strip admirably. In sections that have not been cleared, the staining reaction of the strip is more difficult to observe, but its presence is often indicated by the occurrence in the endodermal cells of contracted protoplasm which runs always tangentially across the cells remaining in contact with the walls in the region of the strip. In fresh sections stained in phloroglucin and then mounted in concentrated hydrochloric acid, the endodermis is usually very conspicuous, this arrangement of the contracted protoplasm giving it the appearance of a continuous dense thread forming a complete ring.

The writer has recently spent a considerable amount of time investigating the behaviour of the primary endodermis (Priestley (12) and (11 c)). Such a cylinder of tissue may be visualised as a chimney in which the bricks are protoplasts while the Casparian strip represents the mortar between the bricks. This cylinder encloses the vascular strands, within which sap is moving through the plant. Organic solutes are frequently present in the sap, which are of the utmost significance in relation to the growth of the tissue of the plant, continued merismatic growth being impossible unless these solutes are freely supplied. The sap contained within the vascular cylinder will diffuse outwards by way of the walls between the protoplasts and will thus reach the endodermal cylinder. Whether it can reach the tissues outside this depends upon the structure of the endodermis. When an endodermis has the primary structure described above the organic solutes are unable to leak out, because the endodermal protoplasts are relatively impermeable and permit very few solutes, and those mainly inorganic, to pass; whilst the Casparian strip appears to be impregnated with fatty substances and is therefore relatively impermeable both to water and to substances dissolved in water. In a stem with such an endodermis then the growth and structural development of the tissues outside the endodermis is severely restricted.

The peculiar appearance of etiolated plants with their elongated thin stems and undeveloped leaves may therefore be attributed in part to the presence of a functional endodermis within them, restricting the supply of sap with nourishing solutes to the tissues within the endodermis. This idea receives remarkable confirmation from a study of the effects produced by illuminating gas or ethylene upon the etiolated epicotyl. The structural effects described immediately suggested that the primary endodermis was no longer being developed and that consequently further growth was accompanied by lateral leakage of the sap into the cortex through the newly differentiated stem tissue near the growing point. The result of the arrival of these solutes in the cortical region of the stem was immediately shown by increase in girth with less development in length, and, as is shown later, the diagetropic curvature might also be anticipated.

The fact that this toxic action of illuminating gas depended upon the unsaturated hydrocarbons present in it was of immediate significance in other investigations in progress. In conjunction with Miss E. North (11 *c*) the writer had arrived at the conclusion that the peculiar properties of the Casparian strip could only be explained on the assumption that it was impregnated with fatty substances. Its staining reactions showed that it was usually also lignified and that it retained acid properties. The difficulty with which the fatty substances were removed, suggested that they were condensation or oxidation products of fats or rather, in view of their acid reaction, of fatty acids. Observations with Mr R. M. Woodman (as yet unpublished) upon the broad bean had shown that the germinating seed contains at the root tip before germination 4 per cent. of fat, and that the fat included both oleic and linoleic acid, though not in sufficient concentration to cause a rapid "drying" of the extracted fat on exposure to air. The peculiar properties of the Casparian strip suggested therefore that the unsaturated linoleic acid present in the root tip, during the tissue differentiation in the region behind the growing tip, diffused from the vascular strand across the cylinder of cells representing the potential endodermis. Here in some way the unsaturated acid was picked out from the saturated acids, and thus concentrated in the walls of a layer of cells abutting upon intercellular spaces containing air, it oxidised and condensed, practically to a varnish, which impregnated the Casparian strip and was responsible for its characteristic behaviour in restricting the diffusion of an aqueous sap. Its sharp localisation at the strip suggested that the diffusing unsaturated acid must be picked up in this region by chemical means, and the acid reaction of the strip indicated that the chemical union was not with the carboxyl group. The significant observations of Neljubow, and of Crocker and Knight, suggest that in the presence of unsaturated hydrocarbons this Casparian strip may fail to form, and thus the structural change in the epicotyl may be produced. The significance of these facts is unavoidable. The unsaturated hydrocarbons diffusing into the root, appear to saturate the chemical linkages which usually pick up the unsaturated fatty acids, so that the latter are no longer held up in the region of the future endodermis.

This argument has been placed first because it preceded in the case of the writer any experimental work with illuminating gas. It only remains to add that experiment and observation support the conclusion thus drawn. Observations were first made upon broad bean seedlings growing in darkness. The epicotyl then develops tall and turgid, round or oval in outline, with a functional primary endodermis reaching to within a centimetre of the growing point. Seedlings were grown over water in bell jars into which a few cubic centimetres of illuminating gas were bubbled. Within three days the epicotyl was evidently swelling in girth as compared with the control. In another day or two the outline of the stem in the "gassed" plant was nearly square in cross section, just as in the normal bean plant grown in daylight. This square stem, the writer had already learnt (11 *f*), to associate with the disappearance of the endodermis. With the help of Miss L. M. Woffenden a complete structural examination of the seedling was made, when it was immediately clear that the base of the swollen region coincided with the appearance of gaps in the functional endodermis, which lower down in the stem formed an unbroken ring, whilst a little further up in the swollen region every vestige of Casparian strip had disappeared. The disappearance of the Casparian strip under gas poisoning has also been seen in the etiolated epicotyl of the pea.

It was interesting to note that the gaps in the endodermis appeared first opposite the spaces between the vascular bundles. This was to be expected as the fatty acids used in the formation of the Casparian strip diffuse outwards from the vascular strands towards the endodermal region. Clearly then these acids will first be anticipated in arrival by the ethylene at points in the endodermal ring which are most distant from the vascular strands.

The observations of Richter (15) upon the histology of the swollen zone of the pea epicotyl are necessary corollaries of the disappearance of the functional endodermis. With the insurgence of the sap from the vascular strands into the cortical regions collenchyma formation follows, whilst it will be shown elsewhere (11 *e*) that the formation of active cork meristems in cortical regions requires the free access to the meristem of the organic solutes from the vascular strands.

The curvature of the etiolated epicotyl or shoot described by

Neljubow (9, 10) and Singer (16) as a diageotropic response and by Molisch (8) and Richter (15) as the combined result of decreased geotropic, and increased heliotropic sensibility, require further examination. The etiolated shoot has always a sharply curved apex which only becomes erect upon exposure to light. The sharp curvature must be an indication of different rates of growth in the two sides of the apical meristem, the inner side of the hook at the apex presumably finding greater difficulty in development. It is only natural, therefore, that differentiation behind the growing apex should also proceed at unequal rates on different sides and that as a consequence the upper rim of the endodermal cylinder should not be at the same level all round the stem. Usually it may be expected to lag behind in development on the side which develops from the inside of the hook. Naturally the failure of the endodermis in the gassed plants usually occurs at a lower level on this side, and extension of cortical tissues follows therefore most rapidly on this side, and the epicotyl is bent over with the hooked apex on the upper side of the horizontal portion. While this is the usual curvature of the horizontal stem it is by no means invariable, the position of the first break in the endodermis is also very irregular and probably determined by a varying combination of internal and external factors.

The explanation just presented of the mechanism of gas poisoning accounts adequately for the sensitiveness of the etiolated epicotyl and the insensitiveness of the normal stem in the same plant, as in the normal stem no functional endodermis is present. It also accounts adequately for the poisonous effect of traces of the gas upon roots of the higher plants where a primary endodermis is invariably present in the growing region. Here again, examination in the case of the broad bean has shown the primary endodermis broken through as a result of the effect of the gas, and the consequent tubercular swelling is clearly due to the abnormal supply of nutrient sap to the cortical tissues just behind the growing point. The importance of the primary endodermis in the development of sap pressures by the root has been emphasised elsewhere (12), and reference to these papers will show that in the opinion of the writer this action of illuminating gas would ultimately prove fatal to the plant, as the supply of sap to the growing aerial portion of the plant depends very largely upon the exudation pressures in the root.

At present the writer is not in a position to discuss the interesting effects of traces of coal gas upon the flower bud of carnation, or the epinastic movement of the leaf of Ricinus, but the effect produced upon cork (p. 148) appears to be closely related to the effect produced upon endodermal development. The mechanism for the normal production of the Casparian strip appears to require two factors: (1) a cell membrane in a specially receptive chemical state; (2) diffusing unsaturated fatty acids. The cell membranes appear to be found in the necessary state only when recently differentiated, and in the presence of oxygen. When cork tissue is forming, the cells are laid down by a meristem, the walls of which can be shown to have many properties in common with those of the meristem at a growing point. As the cells are cut off to the outside they are in contact with oxygen, and at the same time fatty acids are deposited in them and undergo transformation into suberin (Priestley (13), Priestley and Woffenden (11 *c*)). If these acids are unsaturated, then their deposit in these membranes may well be hindered in the presence of gaseous unsaturated hydrocarbons. Under these conditions, the walls would remain unimpregnated with fatty acids, and would be readily distended, giving rise to the proliferated tissue so frequently reported in cases of gas poisoning. The tissue formed under these conditions would be extremely fragile, and as it formed at the base of the normal bark, the strain, engendered by the expanding tissue within, would cause shearing in these weak layers resulting in the development of cracks in the bark. An experimental investigation into the truth of these suggestions is now in progress.

SUMMARY.

1. There is clear evidence in the literature that the toxic action of traces of illuminating gas upon plants may be traced to the presence of gaseous unsaturated hydrocarbons. A concentration of one part of ethylene in ten million of air is toxic to the etiolated epicotyl of a pea.
2. The effect of these unsaturated hydrocarbons can be traced in the case of root or etiolated stem to their inhibition of the formation of a functional primary endodermis, which is usually present in these plant structures.
3. The unsaturated hydrocarbons prevent the formation of a functional endodermis, by preventing the normal accumulations of unsaturated acids in the region of the future Casparian strip.
4. It is suggested that the effect of traces of these gaseous unsaturated hydrocarbons upon cork formation may be due to the arrest of the normal deposit of fatty acids in the membranes of the cork cells.
5. The practical significance of this work lies in the fact that definite diagnostic features may now be sought for when injuries to plants are suspected to be due to gas poisoning.

REFERENCES.

(1) BÖHM (1874). Ueber die Einwirkung des Leuchtgases auf die Pflanzen. *Bot. Zeit.* XXXII, 74–75.

(2) CROCKER, W. and KNIGHT, L. I. (1908). Effect of Illuminating Gas on Carnations. *Bot. Gaz.* XLVI, 259–276.

(3) DOUBT, SARAH L. (1917). Response of Plants to Illuminating Gas. *Bot. Gaz.* LXIII, 209–224.

(4) HARVEY, E. M. (1913). Castor Bean Plant and Laboratory Air. *Bot. Gaz.* LVI, 439–442.

(5) HARVEY, E. M. and ROSE, R. CATLIN (1915). The Effects of Illuminating Gas on Root Systems. *Bot. Gaz.* LX, 27–44.

(6) KNIGHT, L. I. and CROCKER, W. (1913). Toxicity of Smoke. *Bot. Gaz.* LV, 337–371.

(7) KOSAROFF, P. (1897). Einfluss ausserlich Faktoren auf den Wasseraufnahme. *Inaug. Diss.* Leipzig. (Quoted from Harvey and Rose (5).)

(8) MOLISCH, H. (1884). Ueber die Ablenkung der Wurzeln von ihrer normalen Wachstumsrichtung durch Gaze (Aerotropismus). *Sitzungsber. Kaiserl. Akad. Wiss. Wien*, XC, 111–196.

(9) NELJUBOW, D. (1911). Geotropismus in der Laboratoriumsluft. *Ber. der deutsch. Bot. Ges.* XXIX, 97–112.

(10) —— (1901). Ueber die horizontale Nutation der Stengel von Pisum sativum und einiger anderen Pflanzen. *Beih. zum Bot. Centr.* X, 128–138.

(11) PRIESTLEY, J. H. (1922). Physiological Studies in Plant Anatomy appearing in *New Phytologist*, vol. XXI, as follows:

(11 *a*) —— . Introduction.

(11 *b*) —— and ARMSTEAD, DOROTHY. The Physiological Relation between the Xylem and the surrounding Tissues.

(11 *c*) —— and NORTH, EMILY E. The Structure of the Endodermis in relation to its function.

(11 *d*) —— and TUPPER CAREY, ROSE M. The Water Relations of the Plant Growing Point.

(11 *e*) —— and WOFFENDEN, LETTICE M. Causal Factors in Cork Formation.

(11 *f*) —— and EWING, J. Etiolation.

(12) —— (1920). The Mechanism of Root Pressure. *New Phytologist*, XIX, 191–200, and 1922, XXI, 41–47.

(13) —— (1921). Suberin and Cutin. *New Phytologist*, XX, 17–29.

(14) RICHARDS, H. H. and MACDOUGAL, D. T. (1904). The Influence of Carbon Monoxide and other gases upon plants. *Bull. Torr. Bot. Club*, XXXI, 57–106.

(15) RICHTER, OSWALD (1903). Pflanzenwachstum und Laboratoriumsluft. *Ber. der deutsch. Bot. Ges.* XXI, 180–194.

(16) SINGER, MAXIMILIAN (1903). Ueber den Einfluss der Laboratoriumsluft auf das Wachstum der Kartoffelsprosse. *Ber. der deutsch. Bot. Ges.* XXI, 175–180.

(17) STONE, G. E. (1913). Effect of Illuminating Gas on Vegetation. Report of the Botanist. *25th Annual Report Mass Agric. Expt. Station*, pp. 13–28.

(18) WEHMER (1917). Leuchtgaswirkung auf Pflanzen. I. Die Wirkung des Gases auf Sporen und Samenkeimung. *Ber. der deutsch. Bot. Ges.* XXXV, 135–154.

(19) —— (1917). II. Wirkung des Gases auf grüne Pflanzen. *Ber. der deutsch. Bot. Ges.* XXXV, 318–332.

(20) —— III. Wirkung des Gases auf Wurzeln und beblatterte Zweige beim Durchgang durch Erde oder Wasser. *Ber. der deutsch. Bot. Ges.* XXXV, 403–410.

(21) —— (1918). IV. Die Wirkung des Gases auf das Wurzelsystem von Holzpflanzen; Ursachen der Gaswirkung. *Ber. der deutsch. Bot. Ges.* XXXVI, 140–150.

(22) —— (1918). V. Wirkung auf Holzpflanzen; Blausaure als schädlichster Gasbestandteil. *Ber. der deutsch. Bot. Ges.* XXXVI, 460–464.

(*Received March* 30*th*, 1922.)

COMMON SCAB OF POTATOES[1]

PART I

By W. A. MILLARD, B.Sc.

(*Lecturer in Agricultural Botany and Adviser in Mycology, University of Leeds.*)

(With Plates VIII, IX.)

Common Scab has long been one of the most widespread of potato diseases and is recorded as early as 1825. In spite of this, it has hitherto received little attention from mycologists in this country. This neglect may be attributed possibly to the fact that, although the disease occurred in all parts of the country, it was only in certain localities that it appeared with sufficient virulence to make it a serious problem. In such places, as for example, in certain districts in the great potato-growing county of Yorkshire, the crop is sometimes rendered so unsightly by the Scab that it is practically unsaleable. In consequence, many farmers have been obliged to omit potatoes from their rotation on land which, from a yield point of view, was eminently suitable for them.

The distribution and virulence of the disease appear to be closely related to the type of soil on which the crop is grown, occurring chiefly on soil of a light sandy or gravelly nature, to a much lesser degree on heavy soil, and rarely, if at all, on true peaty soils.

Accounts of Common Scab are numerous in American literature, but, in many respects, these appear to be incomplete when applied to the disease in this country and a short description will therefore be given here.

DESCRIPTION OF COMMON SCAB.

The disease first appears in the form of small brown spots on the skin of the tuber. These increase in size and, at the same time, the tissue of the potato immediately below them becomes brown and pulpy. At this early stage, the dark brown surface of the spot remains smooth and unbroken and on it a delicate greyish white mycelium is produced. This rapidly disappears when the potato is lifted and exposed to light.

---

[1] A grant in aid of publication has been received for this communication.

The scabs increase to an indefinite size, two or more often coalescing, but, generally, before a diameter of about 4 mm. has been reached in any individual scab, its covering skin ruptures and a shallow depression is exposed. Very quickly, however, the base and edges of the scab become thickened with layers of cork laid down by the potato in its attempt to cut off the disease from the underlying tissue.

The mature scabs vary considerably in general appearance. In some, the shallow depression formed in the early stages of the disease is never afterwards raised to the surface by the subsequent formation of cork and the affected potato presents a pitted appearance. In others, the scabs are raised by the abundant cork formation and stand out above the surface of the tuber in knob-like projections. These two forms of Scab, which we may call "pitted" and "raised" respectively, appear to be the outstanding types of the disease when it occurs in its most virulent form.

The commonest form of Scab in this country, however, is intermediate between these two extremes (Fig. 1). It is slightly raised and is also characterised by an irregularly concentric series of wrinkled layers of cork arranged around a central core or depression.

These different types, together with others showing greater or lesser variations, will be considered more fully in a later paper.

Cause of Scab. The earliest reference to Common Scab is found in Loudon's *Encyclopaedia of Agriculture* (1825) and reads as follows: "Scab, that is to say, the ulceration of the surface of the tubers, has never been explained in a satisfactory manner. Some attribute it to the Ammonia from the dung of the horse, others to alkali, and certain others to the use of wood ashes in the soil."

In 1884, the idea was introduced by W. G. Smith(1) that Scab was produced by the irritating action of sharp or gritty particles in the soil on the swelling tubers. Much support was lent to this theory by the fact that Scab was certainly most prevalent on soils of a light gritty nature and by the generally accepted belief that ashes produced Scab.

In 1890, however, an organism was isolated in America from a form of Scab known as "Deep Scab" by Thaxter(2) who proved it to be the causative organism of the disease, and gave it the name "Oospora scabies." The nomenclature of the fungus and its place in the systematic scale have since passed through many changes. In 1912, Cunningham(3) placed it in the genus Streptothrix. In 1914, Güssow(4) transferred it to the genus Actinomyces, and Lutman and Cunningham(5) believed it to be identical with *Actinomyces chromogenus* (Gasperini).

Later, however, the work of Krainsky(6), Conn(7) and particularly of Waksman and Curtis(8) showed that the characters of *A. chromogenus* were those of a group rather than of a species. Finally, in 1919, Drechsler(9) made an exhaustive study of the morphological character of the genus Actinomyces and concluded that it should be placed in the Hyphomycetes as a Mucedinous group. He therefore re-named Thaxter's organism *Actinomyces scabies* (Thaxter—Güssow).

Thaxter's original work was only slowly recognised in this country and this was possibly due in part to our ingrained belief in the theory of Mechanical Scab. We may attribute it, however, with more credit to ourselves, to the fact that from Thaxter's own account and the photographs which accompanied it, it was by no means certain that the disease, which he called "Deep Scab" was the same as the "Common Scab" familiar in this country. So much indeed was this the case that Massee(10) in 1910, describes the American Scab and says: "I have but rarely observed this disease in this country."

The writer now realises that Thaxter's "Deep Scab" was the form of Common Scab which he has here named "pitted" and as already has been pointed out, this type of Scab is not the most common in this country. Later American writers have published confirmatory accounts of Thaxter's work in which photographs of scabbed potatoes appear that might well replace our own photograph of typical Scab in Fig. 1. Unfortunately, however, no details of the inoculation experiments appear proving the pathogenicity of the organisms isolated and no photographs of the results of any such experiments. Lutman and Cunningham(11), for example, having isolated species of Actinomyces from scabbed potatoes from various sources, state that these agreed, with a few minor differences, with Thaxter's organism and add "all were found capable of producing scab on inoculation." It seems very surprising that in such an early stage of our knowledge of Scab no details or photographs of these successful inoculations should have been given.

Again, McKinney(12) says: "The writer has studied three strains of the scab organism isolated from scabby potatoes grown in three localities in this state, *all of which* are pathogenic upon growing tubers." No data of the experimental proof of this statement are given, however, although, in this case, the omission is excusable on the grounds that the paper deals only with the nomenclature of the Potato Scab organism.

There appears indeed to be no complete confirmation of Thaxter's work, which he himself called "preliminary" in either American or English literature.

On the other hand, it may be recalled that in 1890, Bolley(13) isolated a bacterium which he claimed was able to reproduce Scab and pointed out that in choosing his material for isolation of the organism, he avoided such scabs as were "blackened or pitted."

Again, in 1915, Pethybridge(14) writing on the "Supposed causes of Brown Scab," refers to Thaxter's organism and says: "Although some writers have gone so far as definitely to ascribe some of our scab to this organism, no scientific evidence appears yet to have been published proving that the said organism is really responsible for our ordinary potato scab."

In 1907, a contribution to our knowledge of the subject was made by Seton and Stewart(15). These investigators showed that Common Scab could not be produced in sterilised soil and this work was followed in 1915 by Pethybridge and Fannin(16) who proved conclusively that the theory of mechanically produced Scab was untenable.

This work received confirmation from some unpublished experiments carried out in 1914 by the present writer, and in 1915 by Mr T. Laycock, then Assistant Lecturer in Agricultural Botany at this University.

In view therefore of the general uncertainty on the subject in this country, it was thought highly desirable to make an *a priori* investigation of the cause of Common Scab and this was carried out concurrently with some work on remedial measures, a popular report(17) of which has already been published.

ISOLATION OF THE ORGANISM OF COMMON SCAB.

This was begun in 1916, and in the subsequent absence of the writer on war service, the primary work of isolation together with the study of the morphological and cultural characteristics of the original cultures obtained was carried out by Miss K. Sampson, then Assistant Lecturer in Agricultural Botany at this University. The writer takes this opportunity of expressing his admiration of Miss Sampson's excellent work in this respect and his gratitude to her for placing her notes and cultures at his disposal on his return.

In the selection of material no choice was made of any particular type of scab, but scabbed potatoes were taken from various samples which came in in the ordinary course of advisory work. No attempt was therefore made in this early work to establish any relation between the type of scab and the culture strains isolated.

The material for inoculation was taken in the majority of cases from young unruptured scabs, but a few mature and ruptured scabs were also used as sources of inoculum. The potatoes were thoroughly scrubbed under the tap, and allowed to dry. The inoculum was then taken from the closed scabs by lifting the covering skin with a sterilised scalpel and removing a small amount of the soft tissue below with a platinum loop. In the case of the open scabs the corky edge was cut away and a scraping taken from the exposed tissue.

The medium used was Potato Agar and the results of the plating are best described in Miss Sampson's own notes on a few of the cases.

Variety of potato	Description of scab	Notes on the plates poured
British Queen	Unruptured	Plate crowded with lichenoid colonies forming a greyish mass over the whole surface and staining the medium dark brown
May Queen	,,	Plate crowded with light grey lichenoid colonies producing a dark brown stain on medium. 12 or more bacterial colonies and 1 mould also present
May Queen	,,	Plate thickly seeded with light brown or grey lichenoid colonies. Greyish brown stain spreading through medium
Great Scot	Ruptured; open	Numerous lichenoid colonies on plate resembling those on British Queen plate

These notes, in themselves, afford strong presumptive evidence that the lichenoid colonies forming so dominant a feature of the plates were those of the causative Scab organism. In appearance, the colonies are glistening and refractive and generally so hard that it is necessary to cut them out from the medium with the platinum loop if transfers are to be made at an early stage of growth. Later, the colonies may become coated with an aerial mycelium (usually greyish white to white on potato agar) from which sub-cultures are easily made. They may be distinguished with certainty from bacterial colonies by examining the plate under the low power of the microscope, when the thread-like and often spiral extremities of their filaments are easily seen.

In all, 10 pure cultures were obtained from the different varieties of scabbed potatoes shown below.

No. of culture	Variety of potato	
1	Dalhousie	
2	British Queen	Isolated from the same scab but showing differences in culture
3	,,	
4	May Queen	
5	,,	
6	Great Scot	
7	,,	Isolated from different scabs on the same potato
8	,,	
9	Witch Hill	
10	,,	

With the exception of No. 1 which was isolated in 1916, all the cultures were isolated in August, 1918. Further examination of the morphological and cultural characteristics of the cultures made it certain that the organisms were members of the Actinomyces group, but, from the first, considerable variations were observed between the 10 strains.

An effort was made to compare them with the different species described by Krainsky(6), Conn(7) and Waksman and Curtis(8), but in no single case could complete agreement be found. This, however, is scarcely surprising since, at the time when this work was being carried out, the study of the Actinomycetes was so recent and the available literature on the subject so scanty that the identification of species was supremely difficult if not impossible. Moreover, it was soon discovered that the characters of any culture underwent considerable variation with age, conditions of growth and with extremely slight differences in the composition and reaction of the media on which they were grown.

Since this time valuable contributions have been made to the subject, on the morphological side by Drechsler(18), and on the cultural side by Waksman(19). The latter, in particular, has now placed the work of distinguishing species on a sound basis by using only media of definite chemical composition and degree of acidity throughout his cultures.

A number of species are thus now clearly defined, but in the case of *Actinomyces scabies* this is still by no means the case.

Thaxter's(2) excellent description was of necessity incomplete. Lutman and Cunningham(11) found what they considered "minor" differences between various strains which they isolated, and, as before stated, placed these strains in the species *A. chromogenus* (Gasperini).

Finally, Waksman(19) in 1919 described a type of *A. scabies* based on his own isolations of the organism but admits that a number of cultures received from other investigators differ very considerably from his own.

In view of this apparent variation in the species or group species, as the case may be, the writer felt no difficulty in considering the 10 strains isolated by Miss Sampson as true *A. scabies*, provided their pathogenicity to potatoes could be proved.

INOCULATION EXPERIMENTS.

These were carried out in unglazed pots 14 ins. in diameter, which were filled with soil to within 2 ins. of the top and sterilised in the autoclave at 130° C. for 1 hour. Under these conditions it was found that the temperature of the soil interior was raised to just over 100° C., which according to Waksman (20) would suffice to kill all Actinomyces present with the possible exception of *A. invulnerabilis*. It might not however preclude some bacterial infection.

The potato sets were sterilised by immersion in one-sixth per cent. Formaldehyde for two hours, and at the time of planting three sterilised glass tubes of ½ in. bore were inserted obliquely into each pot for the purpose of inoculation.

The varieties of potatoes planted and the strain of Actinomyces with which each was inoculated is shown in the following table:

No. of pot	Variety	No. of culture strain
1	Dalhousie	1
2	British Queen	2
3	,,	3
4	,,	1st control—uninoculated
5	Great Scot	6
6	,,	7
7	,,	2nd control—uninoculated
8	May Queen	4
9	Witch Hill	9
10	British Queen	1
11	,,	3rd control—unsterilised soil and tuber. Uninoculated

The first inoculation was made on July 30th when the plants were about 10 ins. high, and two further inoculations at intervals of one month.

The greatest care was taken throughout the experiment to keep the greenhouse as sterile as possible and floor and benches were washed with disinfectant each time the house was entered.

RESULTS OF INOCULATION.

Before tabulating these, a distinction must be made between obvious, typical scabs and spots or points of infection which could only be ascribed to the inoculation after microscopic and cultural examination. These spots appeared to coincide in every case with a lenticel. They were brown to black in colour, of a diameter from 1 to 3 mm. and were very numerous on all the inoculated potatoes. Plate cultures made from the more conspicuous consisted of either all Actinomyces colonies or of Actinomyces and bacteria, and where these were found the spots were considered as definite infections and described as scab spots. Some of the more minute spots, however, which were such as are very commonly found in the lenticels of potato tubers gave bacterial colonies only.

No. of pot	Infection
1, 2, 6, 10	In each of these pots the tubers showed typical scabs from 3 to 6 mm. in diameter. In Pot 2 many of these were covered with the delicate greyish white mycelium so characteristic of the disease. In addition, large numbers of scab spots were present.
3, 5	No mature scabs present, but the tubers were covered with scab spots.
8, 9	These two crops were lifted 5 weeks before the others. No scabs were present. A few spots were shown by the May Queen tubers but these were not examined.
4, 7	Controls. Potatoes clean and glossy. In Pot 7 a few minute spots were present on the tubers but were found to be of bacterial origin only.
11	One or two small but typical scabs of diameter from 4 to 7 mm. were present on most of the tubers. This control pot was included in the series in order to ascertain the extent to which scabbing would occur normally in pots. The soil was known to produce scab badly in the open. It would appear, therefore, from the results that the conditions of pot culture are unfavourable to scab production and this is probably due to lack of aeration. The degree of scabbing produced in the inoculated pots cannot therefore be taken as any indication of the virulence of the Actinomyces strains under normal conditions.

Photographs of the scabbed potatoes from Pots 2 and 6 and of the clean potatoes from the control Pot 4 are given in Figs. 2, 3 and 4 respectively.

From these experiments, therefore, it will be seen that out of the seven strains of Actinomyces tested, five proved to be pathogenic and the negative results given by the remaining two may have been due to the early ripening of the potato varieties (May Queen and Witch Hill) concerned. The positive result in Pot 10, where an Actinomyces strain isolated from a Dalhousie potato was used as inoculum for British Queen potatoes shows that this strain is not specific to one kind of potato and this is probably true of the other strains also.

The experiments thus fully confirm Thaxter's original work and show its applicability to types of scab in this country. They show, in addition, that Common Scab may be produced by various members of the Actinomyces group exhibiting considerable differences in culture. To what extent these different members may be considered as belonging to a single species or species group is however a matter needing further investigation. Some work on the subject is now being carried out and it is hoped to publish the results of this in due course.

The writer wishes to express his thanks and indebtedness to Professor R. S. Seton for the kind interest he has taken in the work, to Mr S. Burr, Demonstrator in Agricultural Botany at this University, for his valuable assistance, and to Mr J. Manby, University Photographer, for the photographs.

REFERENCES.

(1) Smith, W. G. (1884). Text-book. *Diseases of Field and Garden Crops.*
(2) Thaxter, R. (1890). *Ann. Report Conn. Agric. Exp. Stn.* 80–95.
(3) Cunningham, G. C. (1912). *Phytopathology*, II, 97.
(4) Güssow, H. T. (1914). Ref. *Phytopathology*, IX, 327.
(5) Lutman and Cunningham (1914). *Vermont Agr. Exp. Stn. Bul.* 184, 42.
(6) Krainsky, A. (1914). *Centbl. Bakt.* Abt. 2, Bd. XLI, 649–688.
(7) Conn, H. J. (1917). *N. Y. State Agric. Exp. Sta. Tech. Bul.* 60.
(8) Waksman and Curtis (1916). *Soil Sci.* I, 99–134.
(9) Drechsler, C. (1919). *Bot. Gazette*, LXVII, 65–83 and 147–168.
(10) Massee, G. (1915). Text-book. *Diseases of Cultivated Plants and Trees*, 458.
(11) Lutman and Cunningham (1914). *Vermont. Agr. Exp. Sta. Bul.* 184, 24.
(12) McKinney, H. H. (1919). *Phytopathology*, IX, 328.
(13) Bolley, H. L. (1890). Ref. *Ann. Report Conn. Agric. Exp. Sta.* 81 and 84.
(14) Pethybridge, G. H. (1915). *Journ. Dept. of Agric. and Tech. Instr. for Ireland*, XV, 521.
(15) Stewart, J. G. (1907). Univ. of Leeds and Yorkshire Council for Agric. Educ. *Report* 70, 15–16.
(16) Pethybridge, G. H. (1915). *Journ. Dept. of Agric. and Tech. Instr. for Ireland*, XV, 522–4.
(17) Millard, W. A. (1920). Univ. of Leeds and Yorkshire Council for Agric. Educ. *Report*, 118.
(18) Drechsler, C. (1919). *Bot. Gazette*, LXVII, 65–83 and 147–168.
(19) Waksman, S. A. (1919). *Soil Sci.* VIII, 71–215.
(20) —— (1919). *Soil Sci.* VIII, 83.

EXPLANATION OF PLATES VIII AND IX

Fig. 1. A typical example of the commonest form of Common Scab. Reproduced from *Report* 118, Univ. of Leeds and Yorkshire Council for Agric. Educ.

Fig. 2. "British Queen" potatoes—the produce of Pot 2 showing scabs in various stages produced by inoculation with *Actinomyces scabies*, strain No. 2.

Fig. 3. "Great Scot" potatoes from the produce of Pot 6 showing scab spots produced by inoculation with *Actinomyces scabies*, strain No. 7.

Fig. 4. "British Queen" potatoes, the produce of the uninoculated Pot No. 4 (Control).

(*Received March 9th*, 1922.)

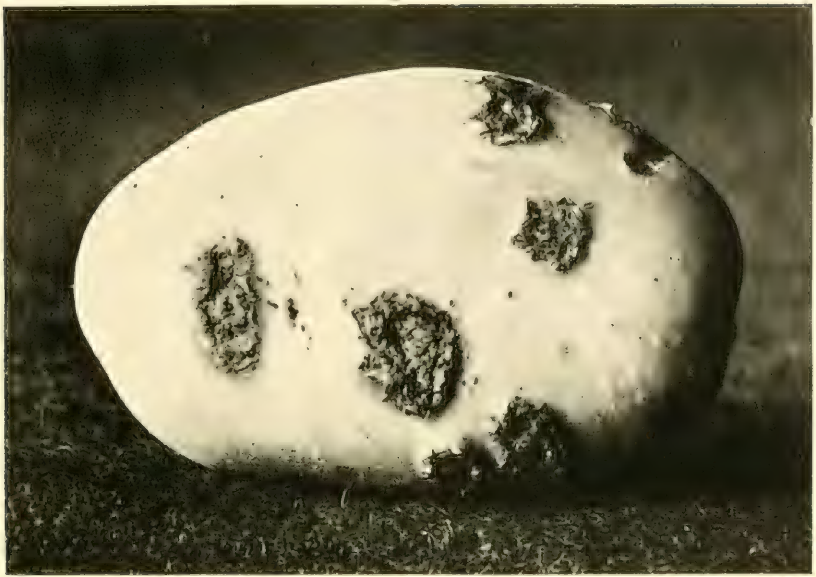


Fig. 1.

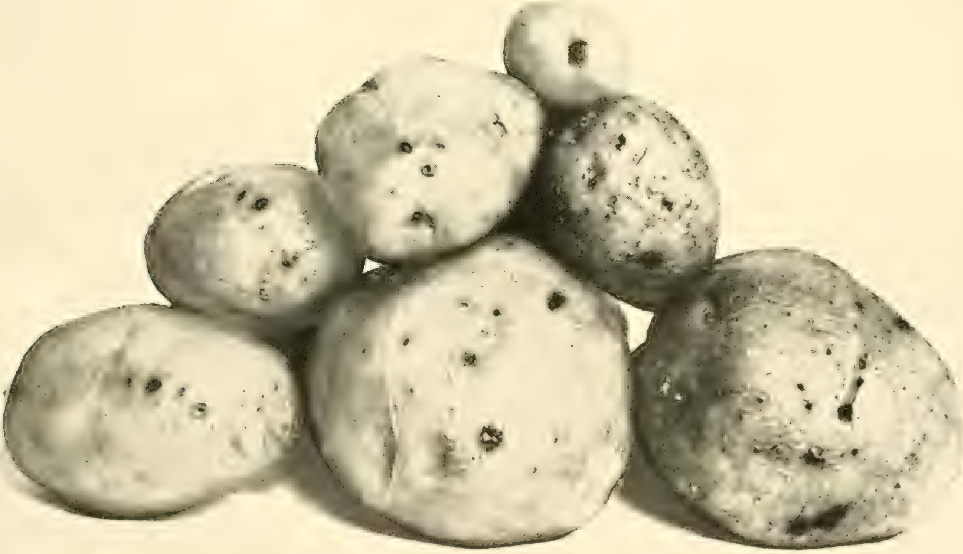


Fig. 2.

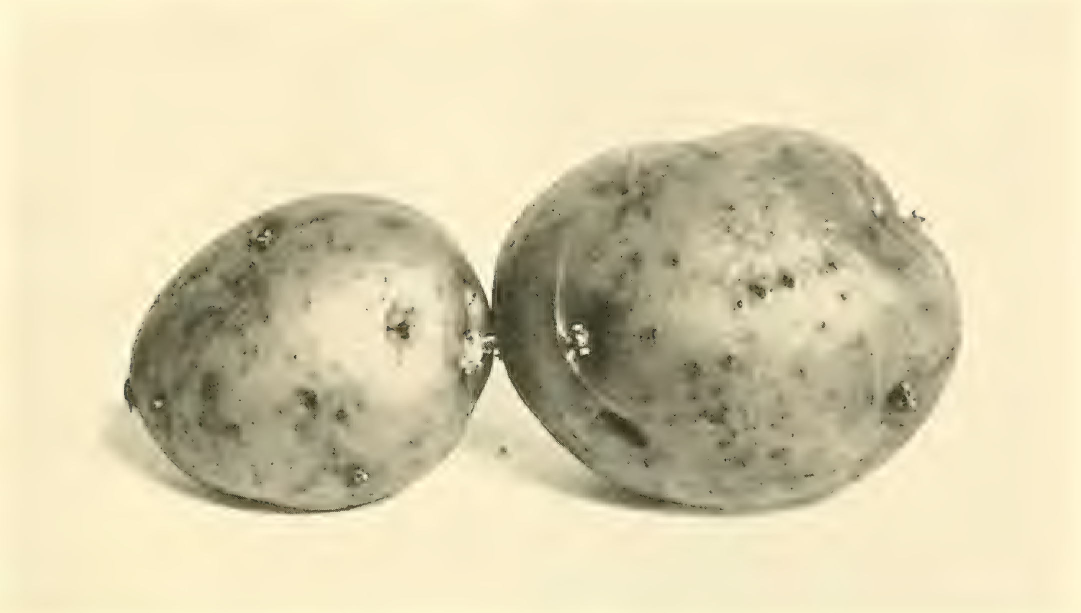


Fig. 3.

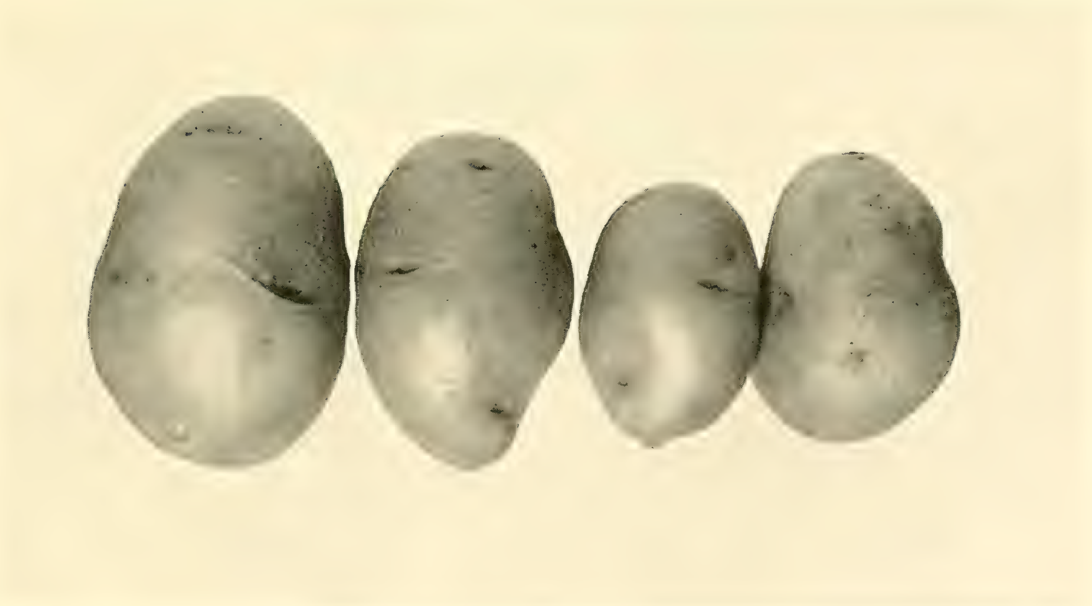


Fig. 4.

ADDITIONAL HOST PLANTS OF *OSCINELLA FRIT*, LINN. AMONG GRASSES.

By NORMAN CUNLIFFE, M.A. (Cantab.),
Christopher Welch Lecturer in Economic Zoology, University of Oxford.

The following observations supplement those recently recorded (1) relating to the utilisation of certain grasses as host plants by *O. frit.* As, in previous experiments, positive results even with the same species of grass were decidedly irregular, it was concluded that the small scale of pot experiments might be unduly influencing reproduction. These flies are difficult to rear, owing to their sensitiveness to unfavourable environmental factors. Therefore in 1920 and 1921 outdoor muslin cages measuring 6′ × 6′ × 4′ were given a trial but, although the conditions were apparently more favourable for oviposition they were not ideal, as emergence figures were still small. In addition to determining which grasses *O. frit* could utilise as host plants, it was desired to obtain some evidence as to the relative preference for different hosts at different periods of the year.

Grasses Utilised in Winter. Experiments I to III.

Briefly the history of the host plants used in the following experiments was as follows: the seeds were sown on 23. iii. 20 under protective muslin cages, the plants being cut down to a height of 4 inches on 1. vi. 20, planted out in 9 inch pots (four roots per pot) on 29. vii. 20 and on the same day recut to a height of 4 inches to induce tillering. The pots were sunk to the brim in a shallow pit under one of the large cages, five pots of each species of grass used being distributed as evenly as possible therein. The parent flies, bred from oat grains, were divided into four lots and introduced through each of the four sides of the cage, water and food (in the form of sugar) being present in the cage in abundance. Thus, as far as possible, the effect of sluggishness on the part of the fly was eliminated, and also the separation of the host plants was facilitated when it became necessary to cage them separately for recording emergence. The small cages were examined at intervals of three or four days, and the newly emerged flies removed. Owing to the small numbers only the total emergence from each species of host plant over each interval is shown below.

Experiment I. The following grasses having given positive results in the winter of 1919 and spring of 1920 were grouped in one cage: (1) *Alopecurus myosuroides*, (2) *Festuca pratensis*, (3) *Lolium italicum*, (4) *Lolium perenne*, (5) *Poa annua*, (6) *Arrhenatherum avenaceum*. These grasses were infected on 16. viii. 20 with 148 flies, the pots being caged separately on 2. iii. 21. *Poa annua* and *Alopecurus myosuroides*, being annuals, had died out by March, 1921. The plants showed signs of attack in the autumn, but the larvae failed to subsist either in the debris or the soil after the death of the host plants.

Only 12 flies emerged in the spring of 1921—from *Lolium perenne*, 1 fly after 287 days and from *Arrhenatherum avenaceum* 1, 2, 5 and 3 flies after 267, 276, 287 and 295 days respectively, reckoning from the date of infection.

Experiment II. Holcus lanatus, *Bromus sterilis*, *Dactylus glomeratus*. *Phleum pratense*, *Hordeum murinum*, *Arrhenatherum avenaceum* var. *bulbosum* and *Avena flavescens* gave negative results in 1919–20, although it has been recorded that *O. frit* may oviposit in spring on these grasses. Having been grouped under one cage, these grasses were infected with 175 flies in 16. viii. 20 and caged separately on 2. iii. 21.

Again a very small number of flies, namely 13, emerged in the spring of 1921, but here they were spread over four host plants. *Holcus lanatus*[1] produced 1 fly after 295 days, *Bromus sterilis* 1 fly after 267 days, *Dactylus glomeratus* 1 fly after 276 days and 2 flies after 287 days, while from *A. avenaceum* var. *bulbosum* 8 flies were obtained, namely 1, 3, 3 and 1 after 267, 276, 287 and 295 days respectively.

The minimum, maximum and mean periods required for the production of the spring generation were 267, 295 and 284 days respectively, the average period being 30 days longer in 1920–21 than in 1919–20, although the dates of infection were practically the same in the two cases.

It is of interest to note that in each of these experiments the majority of the flies emerged from the *Arrhenatherum* species, approximately 90 per cent. being obtained from *A. avenaceum* in the first experiment and 70 per cent. from its variety *bulbosum* in the second.

---

[1] *Exp. III.* Small quantities of the following grasses, grown from seed, were caged separately in August, 1920: *Agropyron repens*, *Agropyron caninum*, *Agrostis alba*, *Aira caespitosa*, *Anthoxanthum odoratum*, *Alopecurus pratensis*, *Bromus mollis*, *Bromus erectus*, *Cynosurus cristatus*, *Festuca pratensis*, *F. sciuroides*, *F. ovina*, *Poa pratensis* and *P. trivialis*. Twenty-five flies were introduced into each cage between Aug. 18th to 31st, but in the spring of 1921 the results were negative. A spare pot of *Holcus lanatus* placed in this series produced one fly on 13. vi. 29.

Previous experiments have shown that *O. frit* may, during the winter period, breed on *Alopecurus myosuroides, Lolium italicum, L. perenne, Hordeum pratense* and *Arrhenatherum avenaceum.* In addition to these therefore, *Holcus lanatus, Bromus sterilis, Dactylus glomeratus* and *A. avenaceum* var. *bulbosum* may be utilised during the winter. Miles(2) states that, in the winter period, he obtained larvae of *O. frit* in *Trisetum* (*Avena*) *flavescens, A. avenaceum, Agrostis stolonifera* (a variety of *A. alba*), *Holcus lanatus* and *L. perenne* in the field, without indicating which host was the most heavily infected.

GRASSES UTILISED IN SPRING. EXPERIMENTS IV AND V.

In the spring of 1921 small quantities of the twenty-five grasses used in the first three experiments were available, as well as a few roots of the following species: *Brachypodium sylvaticum, Avena pratensis, Festuca rubra* and *Hordeum pratense.* Single pots of each of these species were placed under a large cage on 4. iv. 21, infected with 149 flies from the field on 18. v. 21 and separately caged in the middle of June.

Reckoning from the date of infection the emergence of the next generation of flies was as follows: *Agrostis alba,* 2 flies after 62 days; *A. avenaceum* var. *bulbosum,* 2 and 1 flies after 42 and 59 days; *Hordeum murinum,* 12, 7 and 1 flies after 55, 59 and 62 days respectively, while in the same periods *L. italicum* gave 1, 2 and 1 flies only; *Poa trivialis,* 4 flies after 55 days[1]. Thus, *O. frit* may exist on *Agrostis alba, A. avenaceum* var. *bulbosum, Hordeum murinum* and *Poa trivialis* during the spring period as well as in *A. avenaceum, Festuca pratensis, L. italicum, L. perenne* and *Poa annua,* as shown by previous experiment.

A similar experiment conducted in July gave negative results, a period of high temperature occurring during this month, causing the premature death of the parent flies.

Experiment V. In the spring of 1921 the cereals, oats, wheat, rye, barley and maize together with the grass *Lolium italicum* were grouped in the same way as the grasses in the first experiment, five pots of each species being used to ascertain whether the fly in captivity showed any decided preference for oats. The cage was infected on 18. v. 21 with 56 flies from the field and the pots separately caged in the middle of June. The emergence of flies was small, wheat, rye, barley and maize yielding none, *L. italicum* 1 and 4 flies after 50 and 59 days, oats, 1, 1, 1, 4 and 3 flies after 47, 50, 59, 67 and 73 days respectively.

---

[1] A pot of *Secale cereale* included in the group was comparatively heavily infected, 5, 1 and 1 flies emerging after 55, 59 and 73 days respectively.

Experiments IV and V gave minimum, maximum and mean periods of 42, 73 and 58 days (55 flies) between the times of emergence of consecutive generations in the spring, confirming the figures obtained in 1920.

The foregoing records indicate that *O. frit* in captivity does show some preference for *Arrhenatherum* species among the grasses and oats among the cereals, although owing to the small yields no comparative measure of preference has resulted.

Additional observations on the prevalence of the fly in the field, collected during the year 1921 indicate that for the years 1919 to 1921 during which the meteorological conditions were very different, the periods of high and low prevalence tend to be constant. If this conclusion is supported by another season's observations, it will probably render possible an explanation of the fact that early sown crops suffer least damage.

REFERENCES.

(1) Cunliffe, N. (1921). "Preliminary Observations on the habits of *Oscinella frit*, Linn." *Ann. App. Biol.* viii, No. 2, 105–134.

(2) Miles, H. W. (1921). "Observations on the Insects of grasses and their relation to Cultivated Crops." *Ann. App. Biol.* viii, Nos. 3 and 4, 170–181.

(*Received March* 25*th*, 1922.)

STUDIES IN BACTERIOSIS. VI

BACILLUS CAROTOVORUS AS THE CAUSE OF SOFT-ROT IN CULTIVATED VIOLETS

By MARGARET S. LACEY.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

A disease of some considerable importance occurred in the early part of 1921 at the Hayden Violet Grounds, Stourpaine. When received, the plants were in an advanced state of decomposition; the whole interior of the stem was reduced to a soft white mush, and the rot was spreading up the petioles; several of the leaves had already fallen off owing to decay at the base of the petioles, and others were dying from the same cause.

Owing to the advanced state of decay considerable difficulty was experienced in isolating the causal organism, but eventually a pure culture of a white organism which produced vigorous rotting of vegetables was obtained.

Inoculation Experiments upon Cultivated Violets.

Underground stems, "runners," and the apices of stems were used in these experiments. In all cases, rotting of the tissues round the point of inoculation was obtained. In the case of stem infections the rotting proceeded rapidly, after 48 hours the petioles had been attacked and the leaves were dying, after a week a rotted mass from which the leaves had fallen was all that remained. In all cases the controls remained healthy. The organism also produced white rot of carrots, turnips, potatoes and onions.

Identification of the Causal Organism.

The organism was carefully compared with the laboratory culture of *Bacillus carotovorus* (strain obtained from Prof. L. R. Jones in 1911). The latter had an average length of $1{\cdot}2\mu$ and varied from 1–$1{\cdot}6\mu$, whereas the violet strain had an average length of 3μ and varied from 1–4μ with occasional larger cells up to 10μ. Apart from this difference in size the two organisms appeared to be the same, the cultural and physiological features agreed very closely and one has no hesitation in ascribing the disease of violets to that omnivorous organism *Bacillus carotovorus*.

In the first instance the diseased plants were submitted to the Royal Horticultural Society's laboratory at Wisley, where the bacterial origin of the disease was suspected by Mr W. J. Dowson, who referred the matter for further investigation to Dr S. G. Paine to whom the author is indebted for his constant help throughout the work.

(*Received Nov.* 14*th*, 1921.)

REVIEW

Annals des Épiphyties. Edited by MARCHAL, P. and FOEX, E., Tome VII, pp. 1–464 + lxxxvii. Mémoires et Rapports présentés au Comité Supérieur des Stations de Recherches en 1919 et 1920. Ministère de l'agriculture, Paris, 1921. 25 frs.

The volume is planned as in previous years, an interesting summary of the occurrence in France of plant diseases of physiological nature and due to insect, fungus and bacterial parasites occupying pages i–lxxxviii, and brief reports on the activities in the several laboratories of plant pathology and entomology in France filling the last twenty pages. Of the nineteen original papers the first (pp. 1–115) is by G. Arnaud and is a re-grouping into a new family, the Parodiellinaceae of all the Pyrenomycetes possessing internal mycelia with definite haustoria, parasitic especially on the leaves of higher plants. Most of the family have also external mycelia and contain a bright soluble pigment. The genera so grouped include Bagnisiopsis, Parodiellina, Chevaliera, Parodiopsis, Perisporina, Nematothecium, the Erysiphaceae, Exosporina, Septoidium, Ovulariopsis, Oidiopsis and Oidium. The thesis is elaborated in some detail and the memoir, which is illustrated by 25 text-figures and 10 plates, contains much matter of general mycological interest. In pp. 117–167, P. Vayssière gives an account of the campaign against the epidemic of Moroccan locust (*Dociostaurus Maroccanus*) in Crau in 1920. The procedure adopted is described in detail, perhaps the most striking item being the use of "lance-flammes," the programme for the 1921 offensive is outlined and the paper is illustrated by 11 plates. Pages 169–236 are occupied by papers by A. Paillot and by J. Feytaud on the simultaneous control of insect and fungus pests of fruit trees by mixed sprays. Arsenical-lime-sulphur, Bordeaux mixture plus arsenate of lime, Bordeaux mixture plus lead arsenate and lime-sulphur plus lead arsenate are recommended. An interesting paper is contributed (pp. 237–266) by L. Chopard on the biology and control of the Argentine ant (*Iridomyrmex humilis* var. *arrogans*) in the south of France. Pages 267–314 are occupied by a number of papers on potato diseases by H. M. Quanjer, E. Foex, J. Aumiot, E. Blanchard and C. Perret. The chief problems in question are Mosaic disease, Leaf Curl and allied troubles, and views supporting Quanjer's hypotheses and in opposition to them are expressed by the several investigators. J. Feytaud contributes a paper describing the effect of variations of temperature, humidity, etc. on the Eudémis and the Cochylis in the Bordeaux region in 1918 and 1919. In pp. 339–370 P. Vayssière gives an interesting general account illustrated by eight plates of the insect pests of cultivated plants in Morocco, together with notes on Insecticides. Three short papers by R. Régnier describe the entomological research station at Rouen, the damage to Poplar trees caused by the leaf hopper *Idiocerus populi*; and the destructive activities of various species of Corvus in Normandy. Short accounts are given of some biological observations on the olive fly (*Dacus oleae*) and its parasites by R. Poutiers and L. Turinetti, and of the successful employment of chlorpicrin as an insecticide by P. Schindler and B. Trouvelot. There are also papers on fungus diseases of apricots by J. Chifflot and on Fusariose and two other diseases of the melon by J. Dufrénoy. The volume is well produced and is a fine record of work done.

W. B. B.

REPORT OF THE COUNCIL

OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR, 1921–2. PRESENTED TO THE ANNUAL GENERAL MEETING, FEBRUARY 24TH, 1922.

DURING the year eight meetings have been held which have usually been devoted to discussions. The meetings have been well attended, an average of 77 members and friends being present. The following sixteen communications have been made to the Association:

Dr A. S. Horne (Imperial College of Science). "(I) Cultures of Polyopeus. (II) Demonstrations of the Enzymic action of Polyopeus in Solid Nutrient Media containing Starch."

Dr S. G. Paine (Imperial College of Science). "(I) Cultures of the Causal Organism of a Potato Disease. (II) A Novel Method of Inoculation of Potato Tubers."

Dr Wm B. Brierley (Rothamsted Experimental Station). "(I) The Differential Infection of Pure Lines of Wheat by Biological Forms of *Puccinia graminis tritici* (from the Department of Plant Pathology, University of Minnesota)."

Mr G. C. Gough (Ministry of Agriculture). "(I) Potato Tubers Infected Simultaneously by Corky Scab and Wart Disease."

Dr J. Davidson (Rothamsted Experimental Station). "The Cells of Plant Tissues in Relation to Cell Sap as the Food of Aphids."

Mr E. R. Speyer (Lea Valley Research Station). "Ceylon Ambrosia Beetles and their Relation to Problems of Plant Physiology."

Mr Millard (Leeds University). "Green Plant Matter as a Decoy for Actinomyces Scabies in the Soil."

Mr E. H. Richards (Rothamsted Experimental Station). "The Action of Bacteria and Protozoa in Conserving the Nitrogen in Sewage."

Mr Wiltshire (Long Ashton Research Station). "The Methods of Infection of the Apple Canker Fungus."

Mr Engledow (Cambridge Plant Breeding Institute). "The Problem of Increasing the Yields of Cereal Crops by Plant Breeding."

Mr Saunders (National Institute of Agricultural Botany). "Some Problems of Seed Testing."

Dr W. Brown (Imperial College of Science). "The Physiology of the Infection Process."

Dr E. J. Butler (Imperial Bureau of Mycology). "Meteorological Conditions and Disease."

Professor J. H. Priestley (Leeds University). "The Resistance of the Normal and Injured Plant Surface to the Entry of Pathogenic Organisms."

Professor Stebbing (Edinburgh University). "The Importance of Scientific Research in Forestry and its Position in the Empire."

Dr J. Rennie (Aberdeen University). "(I) The Present Position of Bee Disease Research. (II) Polyhedral Disease in Tipula Species."

A Field Meeting was held on July 14th, 1921, when the Association was entertained at Reading by Messrs Sutton and Sons and by Professor Percival and the authorities of the Reading University College.

During the year Mr E. E. Green, finding himself unable to retain his seat on the Council, his resignation was accepted with reluctance and Dr J. Waterston of the Natural History Museum was invited to fill the vacancy. Since the last Annual General Meeting Professor Johannsen of Denmark has been elected to Honorary Membership of the Association and eighteen candidates to ordinary membership. The number of members exclusive of those whose subscription is three or more years in arrears now stands at 240, an increase of nine over last year.

The Laws of the Association have been revised and the amended Laws were published in Part I of Volume IX of the *Annals of Applied Biology*. The issue of the *Annals* has been brought up to date and in future it is hoped to complete one volume each year.

The increase of the annual subscription to the Association to 25*s*., passed by resolution at the last Annual General Meeting, came into force on January 1st of this year.

The Council consider, and are confident that members will agree, that the Association may be congratulated on the progress made during the year as judged whether by the value of the communications received or by the growing membership and the remarkable and increasing attendance at the meetings.

The thanks of the Association are due to Professor J. B. Farmer and his colleagues for granting the use of rooms for the meetings of the Council, and, further, for their unfailing kindness and hospitality in permitting the Association to meet in the Botanical Lecture Theatre of the Imperial College of Science.

OBITUARY

A. W. BACOT

THE death of ARTHUR WILLIAM BACOT, Entomologist to the Lister Institute of Preventive Medicine, took place in Egypt on April 12th last. Bacot had gone out to Cairo, earlier in the year, at the request of the Egyptian Government to investigate problems connected with the aetiology of typhus fever. It is now a well known fact that this disease is transmitted from one human being to another through the agency of the body louse, and great advances have been made in its control by the energetic eradication of that objectionable insect. There are, however, still important links in the chain of evidence with regard to the exact means by which the louse is able to infect man with the disease, notwithstanding the large amount of experimental work which was undertaken during the War. The great prevalence of typhus fever in Egypt rendered it urgent that further investigations should be carried out and, when the choice fell upon Bacot to conduct researches on the entomological aspect of the problem, the Egyptian Government secured the services of an investigator uniquely qualified for the task. Mr Bacot possessed great skill as a manipulator, together with a wide experience of insect-borne diseases, and of typhus fever in particular. These qualifications were coupled with a sterling honesty of character, which led him to subject every conclusion which he formulated to the most rigid tests which he could devise. His work in consequence is of a very high scientific merit, and of quite exceptional trustworthiness.

Bacot was born in 1866, but was not educated for a scientific career, and he became a clerk with a commercial firm in the city. He remained in this capacity for a number of years, devoting his leisure to observing and rearing *Lepidoptera,* which had interested him from an early age. He was never a collector in the ordinary sense: problems relating to the bionomics and getetics of his favourite order mainly occupied his attention, and he contributed various notes and papers dealing with these aspects of his subject. Bacot eventually relinquished his office career and was appointed on the staff of the Lister Institute in 1911. His first researches in medical entomology were concerned with the bionomics of rat fleas, which were undertaken in connection with the work of the Indian Plague Commission. In 1913, in conjunction with Martin, he demonstrated that an important method of transmission of bubonic plague takes place through the medium of the proboscis of the flea. When that insect becomes gorged with the blood of an infected rat, the plague Bacillus increases to such an extent within the gut that the organism forms a mass that plugs the entrance to the stomach of the flea, thereby blocking the passage of the alimentary canal. Further efforts made by the insect at sucking only result in blood being imbibed as far as the oesophagus, and a certain amount of the blood which is taken in flows back into the puncture. Since this blood is freely contaminated with plague bacilli an uninfected host is rendered liable in this way to contract the disease. In 1914 Bacot went out to W. Africa as a member of a Yellow Fever Commission instituted by the Colonial Office. While on this work he made a number of observations on the bionomics of the mosquito *Stegomyia fasciata*, which is the intermediary host of the disease in question. During the period of the War, Bacot was mainly engaged in investigating the body louse and its relation to trench fever. After the conclusion of hostilities he turned his attention to the rôle which that same insect performs in the transmission of typhus fever from man to man. In this capacity he did useful work in Poland. Armed with the experience thus gained, he proceeded along with his colleague Arkwright to Cairo, early in the present year, and there continued to work at aspects of the same problem in the Laboratories of the Institute of Public Health. Unfortunately both men fell victims to the disease not long after their arrival in Egypt. How Bacot became infected does not appear to be known. He was removed to the fever hospital at Abbassia, but succumbed to the malady in rather less than three weeks after the first symptoms appeared. His colleague happily survived and we understand that he is now on the road to recovery. Bacot's funeral took place at the British cemetery, old Cairo, and was attended by a concourse of people, both English and Egyptian, representing many branches of scientific work. His body was borne to its final resting place by friends and colleagues working in the same laboratories.

Bacot's death adds another name to the roll of investigators who have given their lives while endeavouring to solve problems connected with this virulent disease. His place is a hard one to fill, and he has left an enduring name in the annals of medical entomology. The Association of Economic Biologists loses a valuable member, and he had only been elected to the Council of that Society during the present year. Bacot became a Fellow of the Entomological Society of London in 1907, and probably many entomologists recall his last attendance at a meeting, towards the end of last year, when he exhibited microphotographs of the eggs of the European and oriental species of an insect also concerned with the transmission of disease.

A. D. IMMS.

VOLUME IX NOVEMBER, 1922 NOS. 3 & 4

A STUDY OF THE LIFE-HISTORY OF THE ONION FLY (*HYLEMYIA ANTIQUA*, MEIGEN)[1]

BY KENNETH M. SMITH, A.R.C.S.,

Adviser in Agricultural Entomology, Manchester University.

(With Plates X and XI.)

INTRODUCTION.

ERRATUM

The Annals of Applied Biology, Vol. IX, No. 1

Four lines from bottom of page 25

For $\frac{1}{8}$ oz. *read* $\frac{1}{7}$ oz.

Pegomyia cepetorum
Pegomyia ceparum

Hylemyia antiqua, Meig.

The synonyms most commonly in use at present are *Phorbia cepetorum*, Meade, and *Hylemyia antiqua*, Meig. In his *Descriptive List of the British Anthomyiidae*, Meade gives the fly the name *P. cepetorum* and describes quite another species under *H. antiqua*. The *Palaearctic Catalogue of Diptera* and Stein's recent Monograph of the *European Anthomyiidae*, however, give the name as *Hylemyia antiqua*, Meig. It is, therefore, likely that Meade mis-identified this fly or described the same species twice.

It will be more correct in the future to refer to it under the name of *Hylemyia antiqua*, Meig.

---

[1] A grant in aid of publication has been made for this communication.

flea, thereby blocking the passage of the alimentary canal. Further efforts made by the insect at sucking only result in blood being imbibed as far as the oesophagus, and a certain amount of the blood which is taken in flows back into the puncture. Since this blood is freely contaminated with plague bacilli an uninfected host is rendered liable in this way to contract the disease. In 1914 Bacot went out to W. Africa as a member of a Yellow Fever Commission instituted by the Colonial Office. While on this work he made a number of observations on the bionomics of the mosquito *Stegomyia fasciata*, which is the intermediary host of the disease in question. During the period of the War, Bacot was mainly engaged in investigating the body louse and its relation to trench fever. After the conclusion of hostilities he turned his attention to the rôle which that same insect performs in the transmission of typhus fever from man to man. In this capacity he did useful work in Poland. Armed with the experience thus gained, he proceeded along with his colleague Arkwright to Cairo, early in the present year, and there continued to work at aspects of the

Fellow of the Entomological Society of London in 1907, and probably many entomologists recall his last attendance at a meeting, towards the end of last year, when he exhibited microphotographs of the eggs of the European and oriental species of an insect also concerned with the transmission of disease.

A. D. IMMS.

VOLUME IX NOVEMBER, 1922 NOS. 3 & 4

A STUDY OF THE LIFE-HISTORY OF THE ONION FLY (*HYLEMYIA ANTIQUA*, MEIGEN)[1]

BY KENNETH M. SMITH, A.R.C.S.,

Adviser in Agricultural Entomology, Manchester University.

(With Plates X and XI.)

INTRODUCTION.

THE Onion-Fly has become a very serious pest of late years and is widespread throughout the country, more particularly in Lancashire and Cheshire where the observations recorded in this paper were made.

As in the cases of the Carrot and Cabbage Flies, the damage done by the maggots is all to the root and underground portions of the plant. In the larval condition, the food consists chiefly of onions but the maggot occasionally attacks shallots and leeks and has been recorded from Wales as feeding on tulip bulbs.

The pest is so plentiful in Lancashire that in certain districts, especially near large towns, it is impossible to grow onions at all.

SYNONYMS.

The Onion-Fly has been known under many names. The various synonyms include the following:

Phorbia ceparum, Meig.
Phorbia cepetorum, Meade
Pegomyia cepetorum
Pegomyia ceparum
Anthomyia ceparum
Anthomyia antiqua
Hylemyia antiqua, Meig.

The synonyms most commonly in use at present are *Phorbia cepetorum*, Meade, and *Hylemyia antiqua*, Meig. In his *Descriptive List of the British Anthomyiidae*, Meade gives the fly the name *P. cepetorum* and describes quite another species under *H. antiqua*. The *Palaearctic Catalogue of Diptera* and Stein's recent Monograph of the *European Anthomyiidae*, however, give the name as *Hylemyia antiqua*, Meig. It is, therefore, likely that Meade mis-identified this fly or described the same species twice.

It will be more correct in the future to refer to it under the name of *Hylemyia antiqua*, Meig.

---

[1] A grant in aid of publication has been made for this communication.

LIFE-HISTORY.

The Egg. Description. The egg of the Onion-Fly is white in colour and 1 mm. in length. The outer coating is ridged and there is a shallow depression down one side extending about a third of the distance. The egg much resembles that of *Chortophila brassicae*, the Cabbage Root Fly, except that it is larger and the depression is shorter and shallower than is that in the egg of the Cabbage-Fly. Pl. X, fig. 1 shows the egg of the Onion-Fly (A) compared with the egg of the Cabbage Root Fly (B).

Duration of Egg Stage. This period varies according to the temperature. The usual time is about three days but is occasionally prolonged to six or seven days.

The Larva. Description. On hatching from the egg, the young maggot makes its way through the soil and attacks the root of the onion, boring its way in through the base of the bulb. The full-grown larva and the newly hatched larva do not differ materially except in size.

When full grown the maggot is from 9–10 mm. long and 1½ mm. broad at the thickest part. It is white in colour, flattened at one end and tapering to a point at the other. At the broad flattened end which is the "tail," are numbers of tubercles arranged as shown in fig. 2. In the centre of the flattened end are two chitinous projections, these are the posterior spiracles or "breathing pores." Anteriorly, at the "head" end, is a pair of black hook-like "jaws" of strong chitin by means of which the larva bores its way into the onion. These "jaws" are continuous with a chitinous framework to which are attached a number of muscles; surrounding the hooks on the outside of the "head" is a pair of large fleshy lip-like structures. There is also a pair of small papillae. A little further back are the anterior spiracles, these consist of two flattened fan-like outgrowths, one on each side. Each spiracle is composed of eleven finger-like lobes, this number is not constant but varies in different larvae. Fig. 3 is a drawing of the anterior end of the adult maggot and fig. 4 is an enlarged photograph of the whole insect.

Length of Larval Period. From a number of observations made on the length of the larval stage, it was found that the periods ranged from eighteen to twenty-seven days, the average being twenty days. This was in green onions; according to Severin and Severin (1) the larval period is prolonged into four or five weeks in seeded onions of the previous year. Larvae of the later generations living in larger and more mature onions seemed to take longer over that stage than the first generation.

Pupation. When fully grown, the larva leaves the onion and enters the soil to transform into the pupal condition. The exact position in the ground varies but is generally at a depth of two or three inches and may be close up against the onion or a short distance away. On pulling up an attacked plant the pupae may usually be found in the cavity thus created.

Description of Puparium. The puparium is oval in shape, dark brown in colour, occasionally varying to a lighter colour, and 6 or 7 mm. in length. The larval structures are retained and can easily be made out. Fig. 5 is a photograph of the puparium.

Duration of Pupal Period. The following observations were made with numbers of larvae in order to determine the time occupied by the pupal stage.

Larvae pupated	Adult flies hatched	Period
June 20th	July 9th	19 days
,, 23rd	,, 10th	17 ,,
,, 25th	,, 12th	17 ,,
,, 29th	,, 16th	17 ,,
,, 29th	,, 15th	16 ,,

This gives approximately an average of seventeen days for the length of the pupal period.

Description of Adult Fly. The male is a grey insect somewhat like a house-fly in appearance, though rather lighter in colour. Its body is about 6 mm. long and measures $\frac{1}{2}$ inch across the wings. The thorax is of a lighter grey than the rest of the body and has a number of large bristles interspersed with small ones running longitudinally giving the thorax a banded appearance. The abdomen is darker than the thorax and is much more heavily set with black bristles; there is a band of paler grey down the centre of the abdomen. In the male the eyes are very closely set together. The female is very similar to the male in general appearance, except that it is rather lighter in colour, the eyes are widely separated and the abdomen is broader and pointed at the end, owing to the presence of the ovipositor.

The following description of the Onion-Fly under the name *Phorbia cepetorum* is quoted from Meade's *Descriptive List of the British Anthomyiidae.*

"*Head:* face slightly prominent; epistome flat; eyes of male contiguous; antennae of moderate length with the arista thickened and pubescent at its base, but nearly bare in the middle and at the extremity.

Thorax: with the scutellum of a light yellowish-grey colour; the former marked with four indistinct pale brown stripes, and with four rows of black bristles.

Abdomen: oblong and rather narrow, cinereous, clothed with black hairs and showing silvery white reflections when viewed from behind; it is marked down the dorsum with a row of elongated narrow triangular black spots, which form a sub-continuous stripe; the anal segment is grey, small and rather pointed; the sub-anal male appendages are large and hairy.

Wings: hyaline, with the third and fourth longitudinal veins nearly parallel to each other, and the external transverse ones straight, and a little oblique; *Calyptra* and *Halteres* both pale yellow.

Legs: sometimes piceous; hind femora almost bare of hairs or bristles at the base of their under surfaces; hind tibiae of the males furnished with a few short bristles along the middle and upper part of their inner sides. The female is very similar in colour to the male; the eyes are widely separated, the intervening space being red at its front part; the abdomen is dull grey mostly immaculate, conical and pointed at the apex; the calyptra are white and the halteres yellow."

Fig. 6 is a drawing of the female Onion-Fly.

Length of Life of Adult Fly. The writer was unable to determine the length of life of the adult flies under natural condition. In the laboratory, however, they showed considerable longevity. At room temperatures and fed on casein, the flies lived for periods ranging from three weeks to two months.

This may be partly due to the artificial conditions of feeding and the absence of natural enemies, etc. In this connection it is worth mentioning that the flies refused to feed upon sugar and water in captivity but fed readily upon casein.

This is curious when it is considered that the "poisoned bait" method of control, which consists of poisoning the flies with molasses and sodium arsenite, is so largely used. This possibly may be explained by the difference in the sugars used.

Development and Number of Generations. From observations made during the summers of 1920 and 1921, both in the field and in the insectary, it appears that there are three generations of *Hylemyia antiqua* in a season, the third being incomplete. There is no well-marked division between the broods but each one overlaps the other, so that maggots in all stages, puparia and adults are found throughout the summer.

In the unusually hot autumn of 1921, the larvae of the third generation were found attacking autumn sown onions, quite late in October.

The adult flies hatched from overwintering puparia were first noted on the wing on May 8th, though odd specimens have been known to hatch in a mild winter as early as January 25th.

The flies were first observed in the onion fields on May 30th and 31st and in much larger numbers during the early days of June. The maggots of the first generation commenced hatching on the second of June. These had mostly pupated by the end of the month. Flies of the first generation commenced hatching at the beginning of the second week in July and second generation adults were seen ovipositing on August 24th.

The larvae of the third generation have usually pupated by the end of September or the beginning of October though in late seasons they may continue to feed till the end of the latter month.

The third generation thus winter as puparia and the adult flies emerge in the following spring.

Taking forty days as a fair estimate of the duration of the life-cycle from egg to adult, the following table gives an idea of the approximate times of appearance of the generations.

1st Generation
May 28th → Eggs deposited by flies emerged from overwintering puparia
June 1st → Larvae of 1st generation hatch
June 19th → Larvae of 1st generation pupate
July 8th → Adult flies of 1st generation emerge

Approximate Time for Maturation of Flies=7 *days.*

2nd Generation
July 15th → Eggs deposited by 1st generation adults
July 18th → 2nd generation larvae hatch
Aug. 5th → 2nd generation larvae pupate
Aug. 24th → 2nd generation adults emerge

Maturation Period=7 *days.*

3rd Generation
Sept. 1st → Eggs deposited by 2nd generation adults
Sept. 4th → 3rd generation larvae hatch
Sept. 22nd → 3rd generation larvae pupate and hibernate

It should be understood that this table is entirely artificial and does not attempt to do more than give approximate dates for the various appearances. It should also be made clear that the generations are not sharply divided off as they appear in the diagram. Some first generation adults may still be emerging at the same time as the second generation adults; and first and second or second and third generation larvae are often found together.

Habits. Food Plants. The food consists mainly of the onion; the maggots, however, occasionally attack leeks and shallots and have been recorded as feeding upon tulip bulbs and lettuce. Under experimental (1) conditions the larvae have been induced to complete their development in fresh manure and also in radishes.

Injuries Produced. The worst damage to the onion occurs in the spring when the plant is still a seedling. Owing to the small size of the onion and the large numbers of maggots produced, the young plants are devoured wholesale, the larvae migrating from onion to onion, leaving nothing but the green portion above ground. As the onion increases in size, symptoms of attack are yellowing and wilting of the tops which finally lie prone on the ground, the bulb in bad cases being reduced to a rotting semi-liquid mass. Any number of maggots from 3 or 4 to 25 or 30 may be found in one onion bulb.

The maggot, as a rule, enters the onion at the base and works its way upwards, occasionally, however, it enters at the side. Pl. XI, fig. 7 shows onion bulbs destroyed by the maggots.

Reproduction. Oviposition. The eggs are laid on the onion plant, in clusters of half a dozen or more and sometimes as many as twenty or thirty may be found together. They are deposited usually under the thin sheathing leaf surrounding the stem, or in the crutch formed by the outside leaf and the stem. Occasionally the eggs are deposited in cracks in the soil but the more usual procedure is to lay them on the plant itself. The attachment is very slight and eggs found on the surface of the soil beneath the onion have usually been detached by some external agency such as rain or wind.

Pre-oviposition Period. This is an important phase in the life of the Onion-Fly and more so in the light of recent attempts to control this pest by means of poisoned bait intended to kill the fly during the oviposition period.

Sanders(2) puts this period at ten to fourteen days, while Severin and Severin (1) give twelve to sixteen days as the time.

This is a point difficult to determine with any great degree of accuracy owing to the probable effects of the artificial conditions of captivity upon the development of the fly.

From dissections of flies in the laboratory at varying periods from the time of emergence, the writer is inclined to put the time of maturation at a week and sometimes as long as nine or ten days.

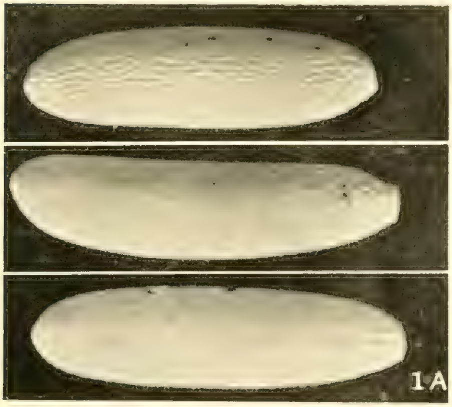
Hibernation. The usual method of hibernation is undoubtedly in the pupal condition. There are cases on record, however, which show that the larvae are also capable of passing the winter.

According to some authors(3) the insect hibernates as an adult but confirmation of this fact is lacking.

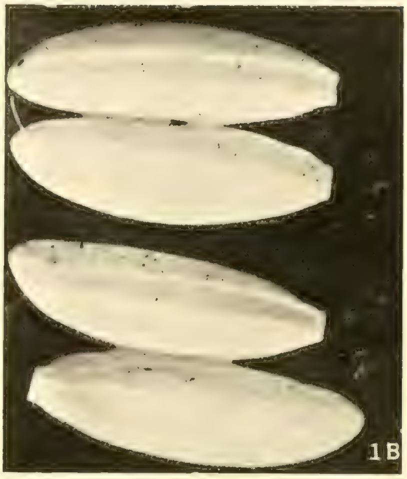
Parasites. One Hymenopterous parasite belonging to the order Braconidae was bred from Onion-Fly pupae, the species being *Aphacreta cephalotes.* This parasite was responsible for largely reducing the numbers of the later generation of Onion-Flies in the summer of 1920. As many as fourteen fully developed adults were dissected from one pupal case of the Onion-Fly.

The parasite also attacks the larvae of *Psila rosae,* the Carrot-Fly. Fig. 8 is a drawing of *Aphacreta cephalotes* and fig. 9 its pupa.

Another useful natural enemy is a beetle *Aleochara bilineata* belonging to the order Staphylinidae or "Rove" Beetles. The larva of this insect



1 A



1 B

4

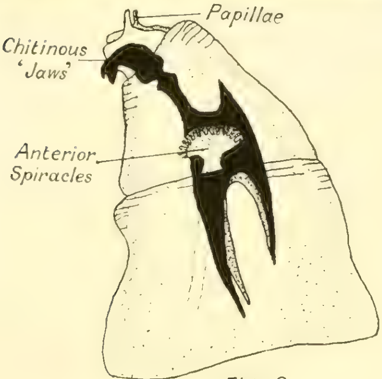

Papillae
Chitinous 'Jaws'
Anterior Spiracles

Fig. 3

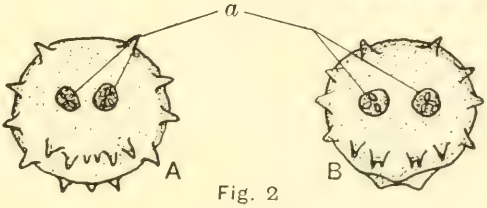


Fig. 2

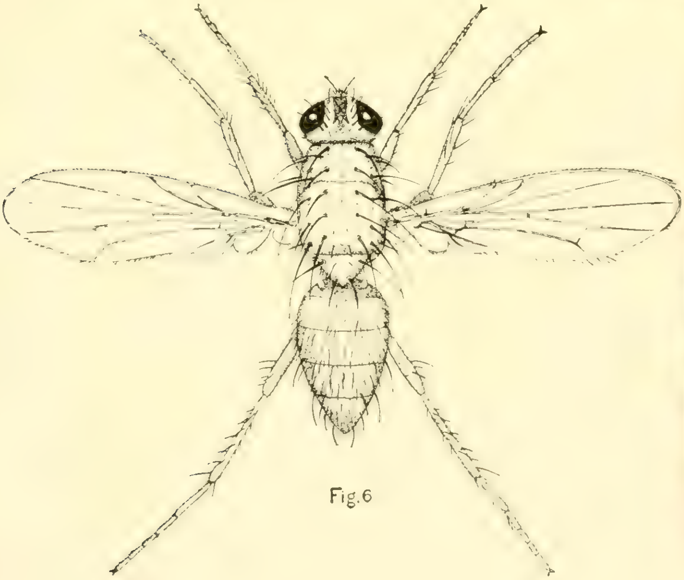
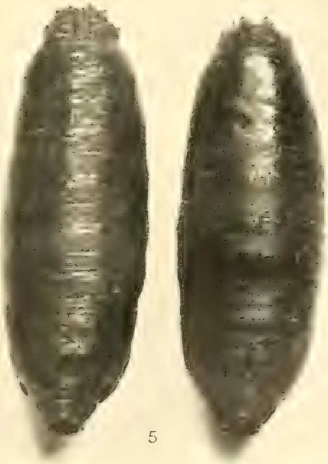
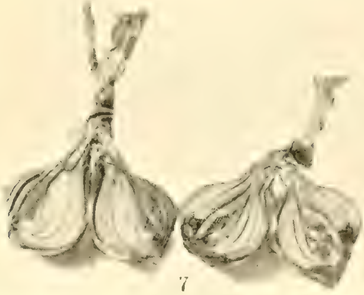


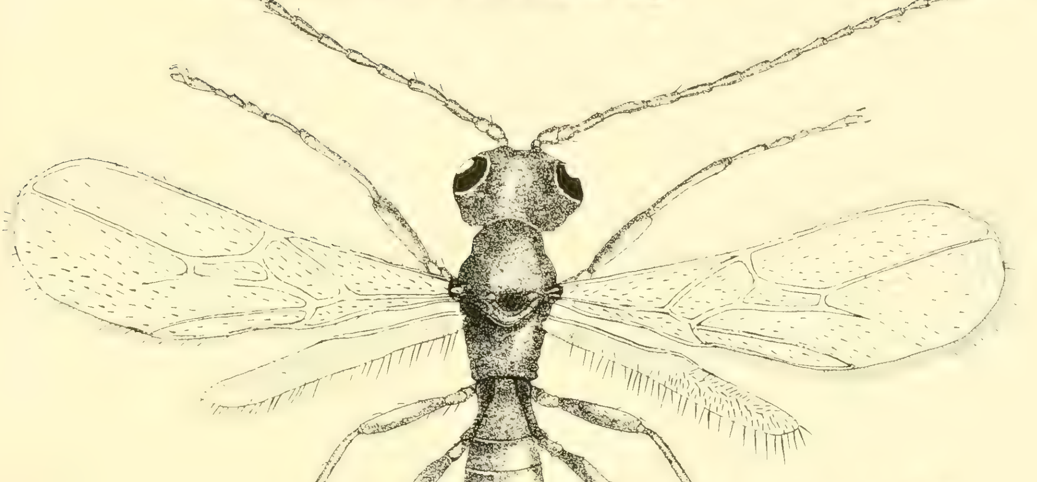
Fig.6



5



7



Ovipositor

Fig. 8

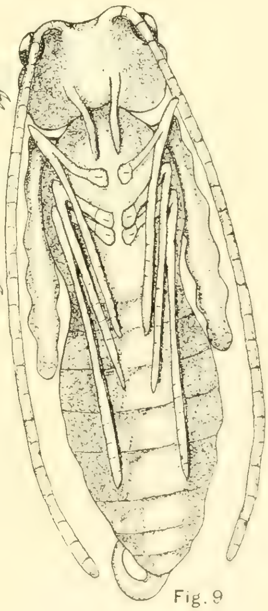
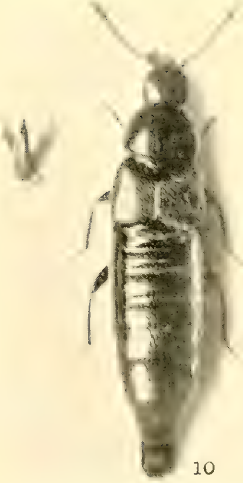


Fig. 9



10

is predaceous upon the pupae of the Onion-Fly, of the Cabbage Root Fly and allied species. This larva bores its way through the hard shell of the pupal case and feeds upon the pupa inside, it then completes its development in the case, emerging later on as the adult beetle. Fig. 10 is a photograph of *Aleochara bilineata.*

Methods. The Onion-Flies were studied in the field, in the open-air insectary and in the laboratory; for the two latter methods large glass cylinders were employed. These were placed over onions growing in pots and the tops were covered with fine muslin. This allowed a clear view of the insects confined within.

Acknowledgments are due to Mrs Tattersall for her kind assistance in preparing the drawings.

REFERENCES.

(1) SEVERIN, H. H. P. and SEVERIN, H. C. (June 1915). *Journal of Economic Entomology,* VIII. No. 3.

(2) SANDERS, J. G. *Journal of Economic Entomology,* VIII. 89.

(3) SMITH, J. B. and DICKENSON, E. L. (Feb. 12, 1907). *New Jersey Agricultural Experiment Station, Bulletin* 200.

EXPLANATION OF PLATES X AND XI

PLATE X

Fig. 1 A. Eggs of *Hylemyia antiqua* compared with
Fig. 1 B. Eggs of *Chortophila brassicae.*
Fig. 2. Posterior spiracles. A. Caudal end of onion maggot. B. Caudal end of cabbage root maggot. (*After* Gibson and Treherne.)
Fig. 3. Anterior end of onion maggot, showing spiracle and chitinous 'jaws.'
Fig. 4. Full grown onion maggot.
Fig. 5. Puparia of onion-fly. Dorsal and ventral aspects.
Fig. 6. Female onion-fly.

PLATE XI

Fig. 7. Onions showing damage caused by the feeding of the maggots.
Fig. 8. *Aphacreta cephalotes.* A parasite of the onion-fly.
Fig. 9. Pupa of *Aphacreta cephalotes.*
Fig. 10. *Aleochara bilineata*, a beetle whose larva is predaceous upon the pupa of the onion-fly.
All except Fig. 7 much enlarged.

(*Received February 9th,* 1922.)

THE SMUT OF NACHANI OR RAGI (*ELEUSINE CORACANA* GAERTN.)

By G. S. KULKARNI.

(With 2 Text-figures.)

This smut was first observed by the writer in 1918 at Malkapur in the Kolhapur State. Later it was collected in the districts of Surat, Nashik, and Ratnagiri in the Bombay Presidency.

The disease is visible only in scattered grains in the head, the majority of grains developing normally. Sometimes the affected grains are single, sometimes grouped in patches of varying size, frequently confined to one side or towards the base or apex of the head.

The sori occur in the ovary as round or occasionally elongated bodies. These project beyond the glumes and they may be from one to six times the diameter of the normal grains (Fig. 1), being often 3–8 mm. in diameter when round, and 4–15 mm. in length when elongated. When fresh their colour is green, occasionally pinkish, but they turn chocolate-brown or dirty black on drying. The colour is due to the membrane, the spore mass being always deep brown to black. On rupture of the membrane the inside is found to contain a powdery black spore mass. The spores are round, 6·6–12·10μ in diameter, dark brown, and have spiny walls.

Germination of the spores occurs easily in nutritive solutions (*e.g.* tomato broth). The spore puts forth a thick, colourless, septate promycelium, and forms spindle shaped sporidia which bud very freely (Fig. 2).

The life-history of the fungus was studied in order to determine whether the disease was seed-borne. A small quantity of Nachani seed was infected with the spores of the smut and was divided into two lots, one lot being then treated with 2 per cent. copper sulphate solution for 10 minutes. The two lots were then sown in separate plots. Smut appeared on a few plants in the plot raised from the infected seed, while in the treated plot all the plants were free from the smut. It appears therefore that the smut is seed-borne and is amenable to copper sulphate seed treatment.

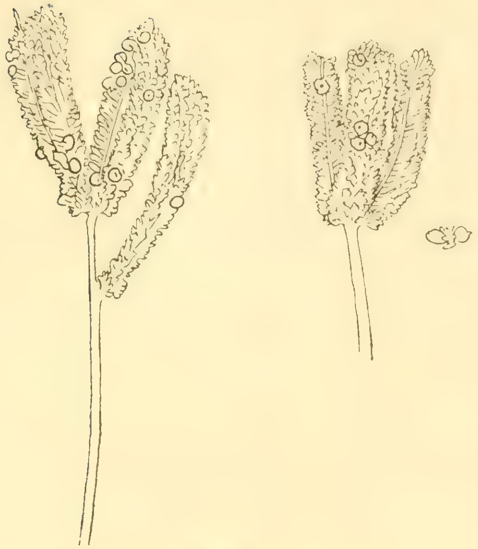


Fig. 1.

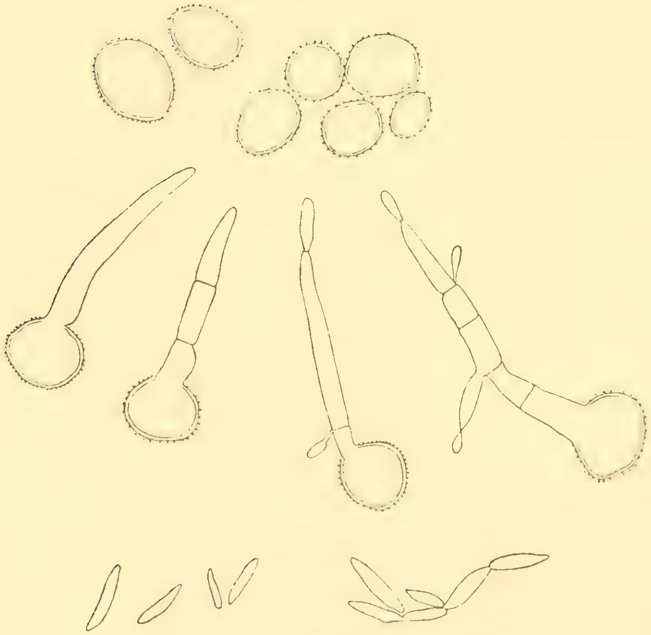


Fig. 2.

The study of the germination of the spores of the smut shows it to be a species of Ustilago, and as no smut of Nachani has been recorded the name *Ustilago Eleusinis* has been proposed, and the following description is given both in English and Latin.

Ustilago Eleusinis nov. sp.

Sori scattered, green or pinkish at first, later becoming darker. Spore mass powdery. Spores round, 6·6–12·10μ in diameter and spiny. Promycelium hyaline, septate, giving rise to many spindle-shaped sporidia.

Habitat: on *Eleusine coracana* at Malkapur in October 1918 in the Bombay Presidency, India.

Ustilago Eleusinis nov. sp.

Sori sparsis, primum viridibus vel roseis, dein fuscescentibus; sporarum massa pulveracea; sporis globosis, 6·6–12·10μ diam. echinulatis; promycelio hyalino septato, sporidiola numerosa fusiformia emittente.

Hab. in ovariis *Eleusinis coracanae* ad Malkapur, in provincia Bombayensi Indiae, Oct. 1918.

(*Received March 4th*, 1922.)

ON THE YOUNG LARVAE OF *LYCTUS BRUNNEUS* STEPH.

By A. M. ALTSON, F.E.S.

(With 2 Text-figures.)

CONTENTS.

	PAGE
Introduction	187
Description of First Instar Larva	187
Description of Second Instar Larva	191
Observations on the Larvae	191
First Instar	191
Second Instar	193
Conclusions	194
Summary	195
Acknowledgments	195
References	195

INTRODUCTION.

THIS paper describes the first and second instar larvae of *L. brunneus*, with some observations on their habits, and it includes a few notes on certain parts of the anatomy of the larvae of later instars.

These observations and notes were made, partly in 1920 and in the following year, during an investigation into the ravages of the beetle, and constitute part of the results, of which some have been published elsewhere.

No account of the early larval stages of any beetle of the genus *Lyctus*, or of the family *Lyctidae*, appears to have been published.

In another paper[1] a description is given of the position of the young larva at the time of maturation, and the observations here are continued from that point.

DESCRIPTION OF FIRST INSTAR LARVA[2].

The first instar larva (Text-fig. 1, I) is creamy white and is very small, averaging 0·65 mm. long by 0·23 mm. wide at the thorax. It is subcylindrical and its body is straight and not arched as the later instars are[1].

---

[1] "The method of oviposition and the egg of *Lyctus brunneus* Steph." (*In the press.*)

[2] Nomenclature after Hopkins (1909, Tech. Ser. No. 17).

The following description is based on specimens mounted in balsam, or glycerine.

The head (Text-fig. 1, 2 A) is broader than thick and is circular viewed dorso-ventrally. It is partially enveloped by the pro-thoracic folds. There are a pair of rudimentary eyes (*e*) composed of pigmented spots and situated below and posterior to the antennae (*a*). No consistency in the shape of the eyes was observed, the number of pigmented spots vary, the majority are in juxtaposition, but a few are some distance apart, and they are deep purple in colour.

(Eyes were found on the larva of each instar[2]. Dugés [1883] in his description of—apparently—the full-grown larva of *L. carbonarius*, refers to a pair of protuberances which he considered to be eyes, and which he figured between the mandibles and antennae. This position differs from that of those of *brunneus*.)

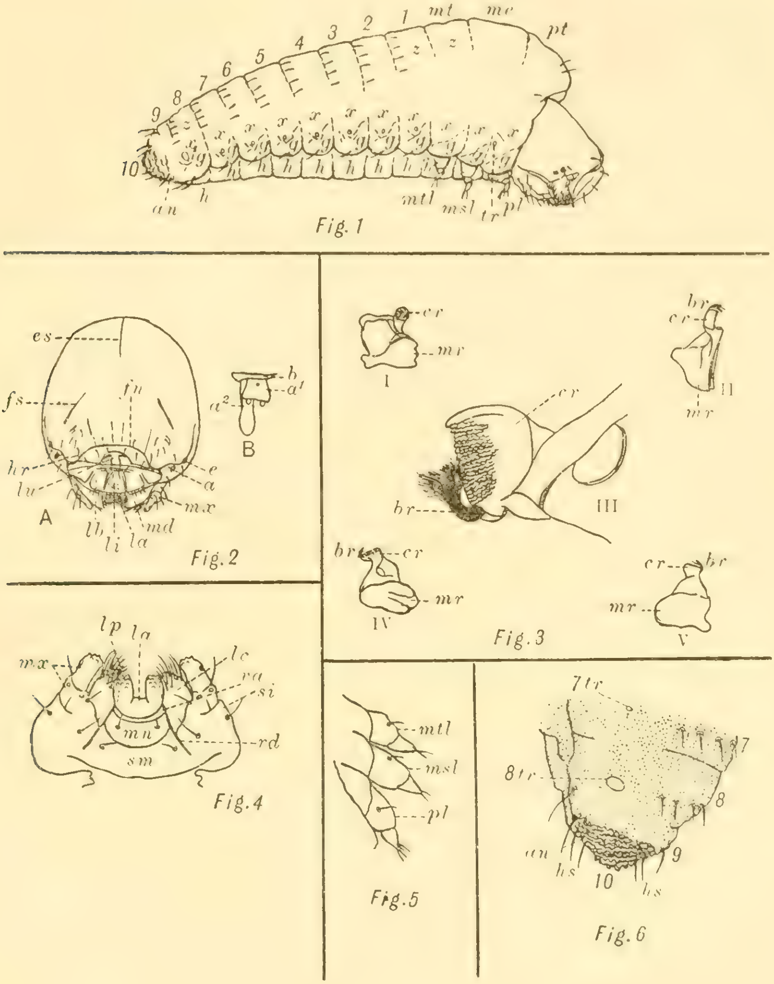
The antennae (Text-fig. 1, 2 B) are telescopic and are situated in recesses and consist of one basal joint (*b*) and two apical pieces (a^1, a^2). One apical piece (a^1), which is the antenna proper, is wider at its apex than its base and terminates in two minute fleshy protuberances, towards its base is a sensory pit. The other apical piece (a^2) is venter and is longer than the dorsal piece. (A ventral apical piece has been found venter to the apical joint of the antenna proper in every instar[2]; in the later stages it decreases in size in an inverse ratio to the size of the apical joint of the antenna, until in the full-grown larva it is barely one-sixth the length.)

The mandibles (Text-fig. 1, 3) are of the same type in each instar and are of a peculiar structure. The molar or distal joint (*mr*) is roughly triangular in outline dorso-ventrally; it is tridentate (Text-fig. 1, 3 IV). Situated dorso-posteriorly and above the molar is an extended dorsal condyle[2], which terminates in a small rounded structure (*cr*) serrated on its inner lateral face; and arising from the outer lateral face of the serrated structure, is a group of chitinised setae (*br*), which are curved round the posterior border to the inner lateral face (Text-fig. 1, 3 III). This extended dorsal condyle which does not appear to bear any relation to the movable *prostheca* of Kirby and Spence (Packard, 1909), is situated at the end of the hypo-pharynx. It comes into operation when the molars are opened and in the act of biting, and their function is, apparently, crushing the food—in the later instars the particles of wood tissue—whilst the setae strain or align it, before it passes into the pharynx.

---

[1] Gahan (1920) in describing the first instar larva of *Anobium punctatum* De Geer states: "At this time they are...straight-bodied, instead of having the body strongly curved as in the older larvae;...." This similarity between these first instar larvae of these systematically closely related beetles, is of some interest.

[2] This is not mentioned by Munro (1915–16) in a description of the full-grown larva.


1
2
3
4
5
6
7
8
9
10
mt
ms
pt
z
x
g
h
an
mtl
msl
tr
pl
Fig. 1
es
fs
fn
hr
lu
A
lb
li
la
md
mx
a
e
b
a¹
a²
B
Fig. 2
cr
mr
I
br
II
III
IV
V
Fig. 3
lp
la
mx
lc
ra
si
mn
rd
sm
Fig. 4
mtl
msl
pl
Fig. 5
7tr
7
8tr
8
an
hs
10
9
hs
Fig. 6

Text-fig. 1.

The labrum (Text-fig. 1, 2 A) is clearly defined and is sparsely fringed with very fine bristles. The clypeus and epistome were not discernible, but the anterior border of the frons (*fn*) is strongly defined and appears as a stoutly chitinised semi-circular rod (*hr*) supporting the upper region of the head. Just below this support is a small semi-circular piece of chitin (*lu*) forming the lumen in which the crushing organ of the mandibles work; between this piece and the anterior border of the frons, a few setae are found symmetrically arranged on either side. The frontal (*fs*) and epicranial (*es*) sutures were barely visible.

The maxillae (Text-fig. 1, 4) consist of two parts, an outer double-jointed palp (*mx*), and an inner piece—the lacinial lobe (*lc*). The stipes (*si*) is present, but the cardo was not distinguishable. The maxillary palp is telescopic, and bears a few scattered setae and sensory pits; the apical joint terminates in five fleshy protuberances. The lacinial lobe bears several stiff setae towards the apex; it is dorsal to the maxillary palp and fused to it; lying venter is the labium (*la*), which is partially defined by chitinised rods (*rd*) supporting it and the maxillae. The labium consists of two single-jointed palps (*lp*) arising from a broad fleshy base, the vaginant membrane (*va*), whose inner surface forms the ligula (*li*), which bears scattered fleshy protuberances; there are a few of these on the apex of each labial palp. The mentum (*mn*) and sub-mentum (*sm*) are clearly defined.

The thorax (Text-fig. 1, 1) is well developed. The pro-thorax (*pt*) partly envelopes the head; and bears a pair of spiracles (*tr*). Each thoracic segment consists of scutal (*x*), scutellar (*z*), epi-pleural (*g*), and sternal (*h*) lobes. Each segment bears a pair of three jointed legs (Text-fig. 1, 5); the pro-thoracic pair (*pl*) are more strongly developed than the others, and terminate in a strong seta surrounded by three longer bristles; the meso- (*msl*) and meta-thoracic legs (*mtl*) each bear two bristles towards their apex.

The abdomen (Text-fig. 1, 1) consists of ten segments (1–10). The first eight are composed of scutal (*x*), scutellar (*z*), epi-pleural (*g*) and sternal (*h*) lobes. There is one bristle on each epi-pleural and a lateral row of four on each scutellar fold towards the anterior border. On each of these segments are a pair of spiracles, those of segments 1 to 7 being of uniform size, whereas the pair on the 8th segment are approximately six times as large as the others; a peculiarity which Perris (1876) considered—in reference to the full-grown larva of *L. linearis* Goeze (*canaliculatus* Fab.)—serves to distinguish the larva of *Lyctus* from all others. No prescutal lobes were observed on the abdomen. The 9th and 10th segments (Text-fig. 1, 6) consist of a series of fleshy protuberances functioning as an anal foot; with the exception of a scutal lobe on the 9th segment, no other lobes were discerned. The anus (*an*) is situated on the 10th segment and is partly enveloped by the hind margin of the 9th segment.

There are several large setae symmetrically arranged on the 9th and 10th segments, which act as "hatching spines" (*hs*).

The larval integument of the thorax and abdomen appears minutely punctured (Text-fig. 1, 6).

DESCRIPTION OF SECOND INSTAR LARVA.

The following description—based on the examination of balsam mounts—is comparative and only points of difference with the first instar larva are referred to.

The second instar larva (Text-fig. 2, 1) is similar in appearance to the later stages, that is, it is arched. It is subcylindrical, and is creamy white in colour, except towards the apex of the abdomen dorsally, where the alimentary tract filled with wood tissue gives it a coloured area, the colour depending upon that of the wood on which it has been feeding. The chaetotaxy is more complex than in the first instar.

The head (Text-fig. 2, 2 A) is slightly rounded from a lateral aspect. The frontal (*fs*) and epicranial (*es*) sutures are fairly pronounced. The antenna (Text-fig. 2, 2 B) differs from that of the first instar, the apical dorsal piece (a^1) is longer than the venter piece (a^2). The trophi are of the same type with a variation in the number of and arrangement of the setae. The eyes (*e*) are more pronounced. The epistomal and clypeal sutures were not discernible.

The thorax (Text-fig. 2, 1) is clearly defined. Each segment is composed of scutal (*x*), scutellar (*z*), prescutal (*o*), epi-pleural (*g*), and sternal (*h*) lobes. The apical joint of the pro-thoracic leg (*pl*) bears six bristles and the terminal seta (Text-fig. 2, 3); the apical joints of the meso- (*msl*) and metathoracic legs (*mtl*) bear three bristles and a terminal seta.

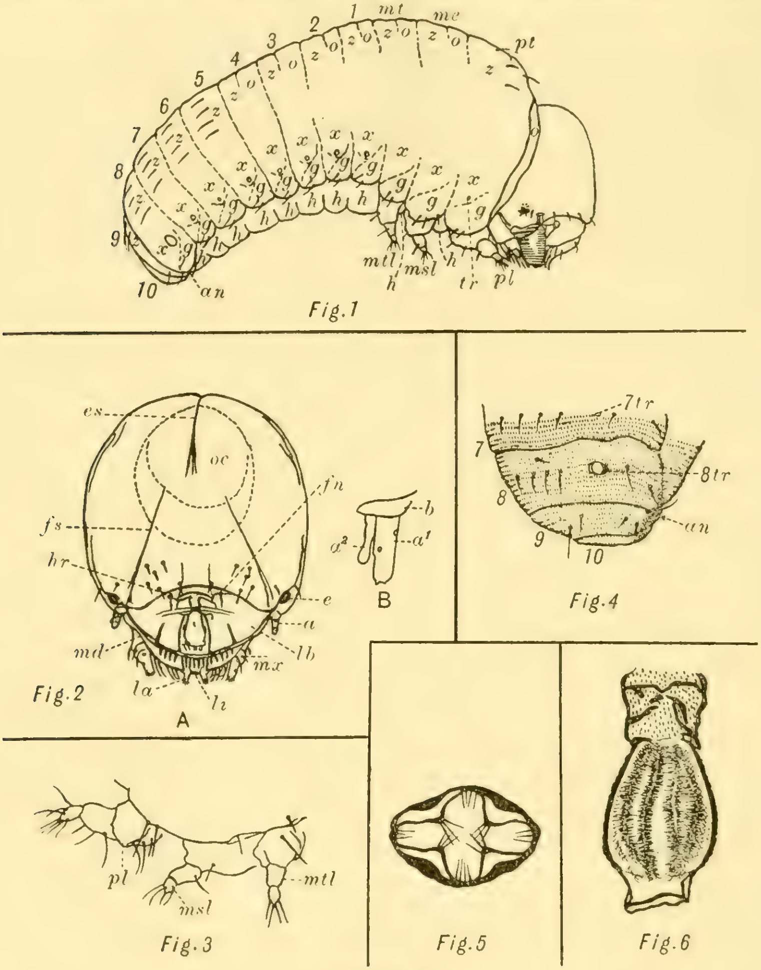
The abdomen (Text-fig. 2, 1). Segments one to four are composed of scutal (*x*), scutellar (*z*), prescutal (*o*), epi-pleural (*g*), and sternal (*h*) lobes; segments five to eight of scutal (*x*), scutellar (*z*), epi-pleural (*g*) and sternal (*h*) lobes. No row of setae was found on segments one to four, but these are present on five to eight. Each epi-pleural fold bears two setae. The 9th and 10th segments (Text-fig. 1, 4) do not bear any fleshy protuberances and no setae appear on the last segment.

The integument of the thorax and abdomen is covered with symmetrically arranged rows of minute chitinised scales (Text-fig. 2, 4).

OBSERVATIONS ON THE LARVAE.

First Instar. As soon as the young larva is fully developed, it commences feeding upon the residual-yolk-mass whilst still enclosed within the chorion, which is soon broken at the posterior pole by its movements and its "hatching spines." Through the aperture thus caused the first particles of excrement pass into the vessel. As the larva progresses it gradually fills the chorion with excrement.

The larva is now travelling along the vessel towards the point of access of its parent's ovipositor. By the time it has eaten its initial food, it has nearly increased in girth enough to fill the vessel, and is able to obtain a grip upon its walls and the necessary purchase—aided by the anal process—to enable it to start attacking the walls and contents of the vessel.


1
mt
me
2
3
4
5
6
7
8
9
10
o
z
pt
x
g
h
mtl
msl
tr
pl
an
Fig.1
es
oc
fn
fs
hr
e
a
lb
md
mx
la
li
A
b
a²
a¹
B
Fig.2
7tr
7
8tr
8
an
9
10
Fig.4
pl
msl
mtl
Fig.3
Fig.5
Fig.6

Text-fig. 2.

At the time of maturation, the mandibles are pale brown and gradually darken as the residual-yolk-mass is consumed, and harden to enable the larva to commence its attack.

In thirteen specific instances it took the young larva from three to five days to consume the residual-yolk-mass. Very little of the vessel contents is eaten, but apparently enough to clear a space for itself to undergo an ecdysis; which was observed to occur between seven and ten days after maturation. Shortly after settling down in the vessel preparatory to moulting, the head capsule is exserted, and the thorax and abdomen become slightly arched.

Second Instar. After extricating itself from the first instar exuvium, which splits primarily along the frontal and epicranial sutures and then along the thorax, the larva rests to harden.

About 24 hours later it commences to bore into the wood tissues.

In the majority of cases observed, the young larva had eaten its way through the vessel, into the tissues, at approximately right angles to its original path, and had taken a downwards course for some distance before again turning at right angles; it had then started boring in a direction opposite to that in which it had originally begun travelling.

In the others, the larva had struck off to the left or right downwards, the direction depending upon which outer margin of the piece of wood the egg had been deposited in. In these cases the larva also turned again after boring some distance down.

In a few instances it was noticed that after biting through the vessel in which it was hatched; it had come into an adjacent vessel, and had turned into this and utilised it for some distance until it had gradually enlarged it and worked its way into the tissues surrounding the vessel.

The foregoing observations are based upon the behaviour of second instar larvae in small pieces of mahogany, of a size which enabled the writer to find the eggs after each piece had been made accessible to females for one night.

The sizes of these pieces, which were split on all faces longitudinally with the vessels, and cut transversely at the ends, ranged from one to two inches in length by one-eighth to about three-eighths of an inch in width and thickness. So that the larvae's movements were considerably confined.

No doubt, in large pieces of wood such as boards, baulks, etc., the larvae would not return in a direction opposite to that in which they had originally travelled along the vessel—after once boring into the tissues—unless they found themselves at the extreme edge or surface of the wood; but would bore in the same direction, only in a lower plane.

The wood tissues, which constitute the food of the larva, are, after passing the crushing apparatus referred to, further broken up in the proventriculus. This consists of longitudinal rows of short chitinised setae. In the full-grown larva there are eight rows (Text-fig. 2, 5, 6), four superior and four inferior.

The proventriculus, oesophagus, pharynx, and the trophi are cast at each ecdysis.

It was observed that the larva's method of boring is partly assisted by the following. (*a*) Its habit of packing the frass behind it into a compact mass by means of continual pressure of the curved apex of the abdomen against the frass; in this, it is aided by the wood tissue contained in its convoluted proctodeum. (*b*) By slightly revolving as it bores, thus enabling it to bite out a bore which is circular in transverse section and in more or less the one plane. (*c*) Its possession of a large quantity of body fluid, which flows rapidly under control and functions in a manner somewhat similar to the body fluid of an emerging Cyclorhaphous Dipteron.

The legs were seen to be used to assist it in revolving, and for clearing out particles of wood tissue, or the frass of another bore into which it had struck. The dorso-lateral thoracic region was observed to fit closely to the bore when distended by the body fluid, but the legs were free to move within the cavity formed by the lateral epi-pleural lobes and the sternum.

CONCLUSIONS.

The rudimentary compound eyes, which are present in all the larval stages of *L. brunneus*, are most clearly defined in the first and second instars. Their presence being probably due to their existence in the remote free-living ancestral larva.

The retention and value of them to a wood-boring larva at first appear obscure, but, when it is remembered that the young first instar larva works its way along the vessel towards the point of access of its parent's ovipositor, to consume the residual-yolk-mass, it is coming towards light, and the surface of the wood; the value of its eyes is obvious. The larva is helpless on the surface of the wood. (None were ever able to get back into a vessel after being placed on the surface.)

In the second instar the value and the use of its eyes are clearly demonstrated by the observations in the foregoing account of the second instar larva's behaviour in the small pieces of wood used in the breeding experiment. For, when it found itself in a vessel at the side it always bored down and towards the centre of the piece, or if in a vessel at either end it bored down and turned on a lower level towards the centre. It is apparent that the rudimentary eyes enable the larvae to remain within the wood. The larva can be said to be negatively heliotropic.

SUMMARY.

1. At maturation, the first instar larva commences to feed upon the residual-yolk-mass contained in the anterior part of the egg, remaining within the chorion to do so. It takes three to five days to accomplish this. It sometimes eats a few particles of the walls or contents of the vessel before settling down to moult.

2. From seven to ten days after reaching maturity the young larva undergoes an ecdysis and then commences its boring operations in the wood.

ACKNOWLEDGMENTS.

The investigation, of which this paper records part of the results, was suggested by Prof. H. Maxwell-Lefroy, Imperial College of Science, to whom the writer has to express his thanks; and to the Committee of the Scientific and Industrial Research Department, for a grant to carry on the work.

The writer is also indebted to Dr C. J. Gahan, Keeper of the Department of Entomology, Nat. Hist. Mus., for identifying specimens of *L. brunneus*; to Prof. Percy Groom, Imp. Coll. Sci., for identifying the various species of timber used in this work.

In addition, thanks are due to Dr A. D. Imms, Rothamsted Experimental Station, for his advice, and assistance in connection with the publication of this paper; and to Prof. S. MacDougall for his efforts to get the original paper published as a whole.

REFERENCES.

BERLESE, A. (1909). *Gli Insetti.* Milan.

DUGÉS, E. (1883). Métamorphoses du *Lyctus planicollis* Le Conte, *Ann. Soc. Ent. Belg.* Tome XXVII. pp. 54–58, plate I.

GAHAN, C. J. (1920). *Furniture Beetles, their Life-history and how to check or prevent the damage caused by the Worm.* Brit. Mus. (Nat. Hist.) Econ. Ser. No. 11. London.

HENNEGUY, L. F. (1904). *Les Insectes.* Paris.

HOPKINS, A. D. (1909). *Contributions toward a Monograph of the Scolytid Beetles. I. The Genus Dendroctonus.* Tech. Ser. No. 17, Part 1, U.S. Dept. Agric., Bur. Ent., Washington, D.C.

Hopkins, A. D. (1909). Some Insects injurious to Forests. Insect depredations in North American Forests. *Bull.* No. 58, p. 66. Part V. U.S. Dept. Agric., Bur. Ent., Washington, D.C.

Munro, J. W. (1915–1916). The larvae of the Furniture Beetles. Fams. Anobiidae and Lyctidae. *Proc. Roy. Phys. Soc. Edinburgh*, xix. Parts Nos. 8 and 9.

Packard, A. S. (1909). *A Text-book of Entomology.* New York.

Perris, Ed. (1876). Larves des Coléoptères. *Ann. Soc. Linn. de Lyon*, Tome xxiii. pp. 60–63, figs. 247–250.

Schiodte, J. C. (1861–83). *De Metamorphosi Eleutheratum Observationes: Bidrag til Insekternes Udviklingshistorie.* 12 parts. 3 vols. Copenhagen.

Xambeu (1898). Mœurs et Métamorphoses du *Lyctus canaliculatus* Fabricius. *Bull. Soc. Sci. Nat. l'Ouest France*, Tome viii. pp. 69–72.

EXPLANATION OF TEXT-FIGURES 1 AND 2

REFERENCE LETTERING.

a, antenna; a^1, a^2, apical joints of antenna; *an*, anus; *b*, basal joint of antenna; *br*, brush-like group of setae on crushing organ; *cr*, crushing organ of mandible; *e*, eye; *es*, epicranial suture; *fn*, frons; *fs*, frontal suture; *g*, epipleural lobe; *h*, sternal lobe; *hr*, semicircular rod of chitin supporting upper region of head; *hs*, hatching spines; *la*, labium; *lb*, labrum; *lc*, lacinial lobe; *li*, ligula; *lu*, piece of chitin forming lumen for crushing organs; *md*, mandible; *me*, mesothorax; *mn*, mentum; *mr*, molar or distal joint of mandible; *msl*, meso-thoracic leg; *mt*, metathorax; *mtl*, metathoracic leg; *oc*, occipital foramen; *pl*, pro-thoracic leg; *pt*, pro-thorax; *rd*, rod; *si*, stipes; *sm*, submentum; *tr*, spiracle; *va*, vaginant membrane; *x*, scutal lobe; *z*, scutellar lobe; 1–10, abdominal segments.

TEXT-FIG. 1.

1. First instar larva. Camera lucida × 80.
2. (A) Dorsal aspect of head. C.l. × 128.
   (B) Lateral aspect of antenna. C.l. × 177.
3. Mandibles: (I) Latero-anterior; (II) Dorsal; (III) Crushing organ and brush of full-grown larva; (IV) Inner lateral; (V) Outer lateral. C.l. × 132.
4. Ventral aspect of maxillae of 2nd Instar larva. C.l. × 177.
5. Legs of larva, left. C.l. × 132.
6. Apex of abdomen, segments 7–10. C.l. × 132.

TEXT-FIG. 2.

1. Second instar larva. Camera lucida × 80.
2. (A) Dorsal aspect of head. C.l. × 128.
   (B) Lateral aspect of antenna. C.l. × 177.
3. Legs of larva, left. C.l. × 132.
4. Apex of abdomen, 8–10, and part of 7. C.l. × 128.
5. Transverse section of proventriculus of full-grown larva. C.l. × 80.
6. Optical longitudinal section of proventriculus of full-grown larva. Drawn from photomicrograph. × 80 (approx.).

(*Received May 26th*, 1922.)

EFFECT OF HIGH ROOT TEMPERATURE AND EXCESSIVE INSOLATION UPON GROWTH.

By WINIFRED E. BRENCHLEY, D.Sc.

(*Rothamsted Experimental Station.*)

ASSISTED BY

KHARAK SINGH, M.A.

(*Punjab Agricultural College, Lyallpur.*)

(With 2 Text-figures.)

In an earlier paper (3) it was demonstrated that the reduction of light due to the over-crowding of barley plants brings about a condition of light starvation which has a harmful effect upon growth, even when an abundance of food and water is supplied to the roots. The suggestion was made that this factor of light competition might be equally or even more important in the case of broad-leaved plants, as greater overshadowing might occur.

To test this water culture experiments were repeated several times with peas at different seasons, 64 plants being closely crowded in a solid square, and 64 others having abundant room to prevent any shading of one another. The nutrient solutions were changed frequently and the tendrils of the peas were cut off as early as possible to prevent damage from one plant clinging to its neighbour when being moved.

Sutton's[1] Harbinger peas were used throughout.

In a test carried on from September 10th to December 21st, 1920, the prevailing conditions were:

Average weekly maximum temperature of house	9–26° C.
,, ,, minimum ,, ,,	2–11° C.
Total hours of sunshine per week	45·9–48

Temperature and sunlight both fell off considerably during the latter half of the experiment.

---

[1] We are indebted to Mr Martin Sutton for the gift of all the seeds used in these experiments.

From a comparatively early date the advantage seemed to be with the spaced plants, and became more marked as growth proceeded and the intensity of light decreased with the waning season. The difference in shoot growth was not noticeable for several weeks, but the roots of the spaced plants soon became strong and bunchy, being larger than any of the crowded roots. In the latter the roots on the outside were comparatively strong, but decreased steadily in size towards the middle of the square, where they were fairly long but very thin. At harvest-time the spaced plants were strong and healthy, well branched, bearing plenty of long well-filled pods, while the roots were very strong. In the crowded square, on the other hand, the middle plants were obviously smaller in all respects than the outer, the difference being now as noticeable in the shoots as in the roots. Most of the pods were thin and distorted, and the seeds had not developed properly.

Table I.

Dry Weights. (Mean figures.)

	Shoot	Root	Total	Efficiency index
Spaced plants:	3·488 ± ·081	·474 ± ·016	3·962 ± ·095	2·253 ± ·024
Crowded plants:				
Outer rank	2·478 ± ·057	·332 ± ·009	2·810 ± ·066	1·953 ± ·022
2nd	1·726 ± ·093	·236 ± ·011	1·962 ± ·102	1·609 ± ·046
3rd	1·348 ± ·055	·173 ± ·010	1·521 ± ·065	1·402 ± ·041
Inner	1·574 ± ·061	·207 ± ·012	1·781 ± ·072	1·556 ± ·039

The above table shows how seriously the reduction of light due to overcrowding affected the growth of peas. A large reduction in dry weight and efficiency index occurred at the outer edge of the square, although one side of each plant was free from light competition, and this reduction was intensified inside the square, where shading came on all sides. Apart from the outer row the differences between the plants were not very marked, showing how effective is the shading of pea plants by their neighbours when in close proximity. Broadly speaking, these results are comparable with those obtained for barley, and indicate a similar reaction of broad- and narrow-leaved plants to light deficiency. The percentage of nitrogen in the spaced plants was lower than in the crowded ones, being only 3·62 per cent. against 4·15 per cent. As with barley this probably shows that peas utilise less nitrogen in the production of each unit of dry matter when adequate illumination is available.

When, however, the above experiment was carried on under conditions of very high temperature and prolonged intense sunshine, certain differences in behaviour manifested themselves which demanded closer investigation.

Between May 7th and June 25th, 1920, the following conditions prevailed:

Average weekly maximum temperature	27–35° C.
,, ,, minimum ,,	8–13° C.
Total hours of sunshine (per week) ...	49·3–65·7
Daily average hours of sunshine ...	8·4

It was soon evident that the crowded plants were making the better growth; they were larger and greener than the spaced plants, some of the latter becoming yellowish, with leaves that inclined to shrivel. The crowded peas maintained their apparent lead, and when cut were mostly healthy and green, with only four casualties, whereas many of the spaced plants were pale in colour and 15 out of the 64 had succumbed.

The effect of competition was evident in the crowded square, as the outer plants averaged 2·127 ± ·065 gm. and the average of the inner ranks varied from 1·523 ± ·185 to 1·686 ± ·058. The spaced plants, however, failed to demonstrate the advantage of the extra light they had received, as their mean weight was only 1·912 ± ·042 gm., less than the outer rank of the crowded set.

A marked difference was noticeable between the plants in the spaced set. Those which were green and healthy had good stiff roots studded with rather outstanding sturdy laterals, whereas in those in which the upper leaves were turning pale the roots looked unhealthy and brown, and were flabby and inclined to be slimy. The worse the shoot the worse the root. The green healthy plants were of the normal type, with one tall shoot and large dark green leaves, whereas those with pale shoots were bushy at the base, owing to the development of axillary buds. This was apparently due to an effort to overcome some detrimental factor acting upon the spaced peas and preventing them from developing normally, for in the earlier experiments with barley the crowded plants also appeared to make the larger growth on the whole, but were not so heavy as the spaced plants when cut. Even the outer rank of the crowded plants showed the influence of this adverse factor to some extent, as the mean weight was not very much above that of the inner plants which were under the influence of more light competition. As in the first experiment described the plants within the square were all very similar in growth and weight.

The harmful effect was obviously due to the prevailing high temperatures or the excessive power of the sun's rays, or both, but the relative importance of these two factors was by no means clear. For several years it has been noticed that plants fail to do well in the greenhouse in hot summer weather, whereas the same species flourish outdoors at the same time, and it was suspected that the high temperatures reached by the culture solutions had some connection with this phenomenon (4). An examination into temperature conditions was therefore undertaken.

In the last experiment described (p. 199), temperature readings of the nutrient solutions taken on various occasions on hot days showed very considerable differences according to the situation of the plants. Two typical records were as follows:

	Air temperature (shade)	Crowded plants		Spaced plants
June 7th, 2.30 p.m.	23° C.	Outer rank	19° C.	25·5° C.
		2nd ,,	16° C.	
		3rd ,,	15·5° C.	
		Inner ,,	15·5° C.	
June 25th, 10 a.m.	24·5° C.	Outer rank	20° C.	22·5° C.
		2nd ,,	18·5° C.	
		3rd ,,	18·5° C.	
		Inner ,,	18·5° C.	

On hot sunny days, therefore, the spaced plants were liable to be subjected to very high temperatures at the root, on occasion exceeding that of the air. In the crowded square, however, the outer ranks received a partial shelter from their neighbours and the solutions never became so hot, while within the square all the temperatures were usually very even, within a very few degrees, and were somewhat lower than the others. Under these conditions the crowding apparently served as a measure of protection either by keeping down the root temperature or by the reduction it effected in the amount of sunlight reaching the leaves. It is obvious that beyond a certain limit the effect of high root temperatures and of abundant sunlight became directly harmful and inhibited growth, but the extent to which each factor was responsible was not shown by the data obtained.

Further knowledge on this point was gained from a similar experiment carried on in the abnormally hot autumn of 1921, when readings were made of the daily maximum and minimum temperatures of the solutions of specified plants. No shading was applied to the greenhouse, and the sun's rays struck through the sloping roof directly on to the crowded square and some of the spaced plants, while the rest of the latter were on a side bench under a higher roof at a different angle, from which the concentration of the sun's rays seemed to be considerably less, though the light intensity was apparently not affected. The environmental conditions were as follows:

Average weekly maximum temperature of house	23·6–31° C.
,, ,, minimum ,, ,,	10·0–15·3° C.
Total hours of sunshine (per week)	21–52
Daily average hours of sunshine	5·8

In this case the crowded plants showed less difference among themselves than usual, the outer ones averaging 1·681 gm. against 1·339 gm. for the inner. The spaced plants alongside were seriously harmed, and only produced 1·055 gm. dry matter.

The temperature records of the solutions were:

	Highest max.	Lowest max.	Mean max.	Highest min.	Lowest min.	Mean min.	Diff. between daily max. and min.
A. Spaced (under sloping roof)	29·5° C.	16° C.	26° C.	17° C.	8° C.	13·5° C.	21–2° C.
B. Corner of square	29·5° C.	15° C.	22° C.	17° C.	8·5° C.	13·5° C.	15·5–1·5° C.
C. Middle of square	23·5° C.	14° C.	18·5° C.	20° C.	12° C.	16° C.	6·5–0·5° C.

In these spaced plants the mean maximum temperature was very high, and for a period of seven successive days the solutions ran up to above 29·7° C., the highest reading of the thermometer. The differences between the day and night readings were therefore often large, although the mean minimum did not fall below that of the outer crowded square. A surprising difference was evident with the spaced plants on the side bench. These grew well and strongly, looked better than any of the crowded plants, and when cut averaged 2·171 gm. dry matter against the 1·055 gm. of the spaced set under the more sloping roof, *i.e.* they were twice as heavy. Daily temperature records were not taken for this set, but on several occasions readings were made of all the solutions, and they were always approximately the same for both sets of spaced plants.

	Oct. 5th, 2 p.m.	Oct. 6th, 2 p.m.	Oct. 11th 12 noon
Spaced, on side bench	31° C.	28·5° C.	24° C.
Spaced, under sloping roof	31° C.	28·5° C.	25° C.
Air temperature	28·5° C.	28° C.	26·5° C.

The relation of temperature to growth has been considered by various workers, but generally in connection with the rate of growth of the roots of seedlings during short periods covering a few hours at most. Under these conditions Leitch (7) found that for peas 30° C. is a critical temperature above which growth is adversely affected, 28–30° C. being the optimum, considered as the highest temperature at which no time factor is operating. Lehenbauer (6), working with maize in water cultures, showed that the optimum temperature varied with the period of exposure, and that with prolonged exposure to the initial optimum the rate of growth falls off rapidly. It may therefore be concluded that the higher temperatures near the optimum for short exposures exercise an adverse influence when they continue to act throughout the life of the plant. Balls (2) attributed the decrease and ultimate cessation of growth at high temperatures to the accumulation of katabolic products in the cells, prolonged exposures to submaximal temperatures favouring the rapid production of these substances.

In the experiments under consideration the initial optimum of 30° C. for peas was exceeded on nine occasions, most within a single week, the highest maximum reaching 34° C. These air temperatures were only maintained for a short time, at the hottest time of the day, the period of exposure being thus very short and rare in occurrence. The average maximum temperature ruled several degrees lower, except for the one week. Furthermore the diurnal fall to the minimum temperature was considerable, 10–15° C. or more, and, as Askenasy (1) and Leitch (7) have both demonstrated that the rate of growth follows immediately and accurately any considerable change of temperature, the slowing off of growth would permit of the reduction of accumulated katabolic products and mitigate the effects of exposure to high temperatures. It would seem, therefore, unlikely that the temperatures, *per se*, were high enough to be harmful to growth, as almost the whole of the air temperature curve fell below 31° C., the initial optimum for short period exposures, especially as the root temperatures during the same period were on the whole rather lower, though they followed the air temperatures fairly closely. The adverse factor is to be sought in the intensity of the sun's rays —much depression of growth occurring where they were focussed on the leaves under the sloping roof. The different angle of incidence of the rays on to the side bench prevented such undue concentration on the leaves, and growth was correspondingly better under similar temperature conditions. This is further corroborated by comparison with the May–June (1920) experiment. In both cases the mean weekly temperatures were very similar, as the higher summer maxima were almost compensated for by higher autumn minima. The May–June plants received far more sunshine—411 hours against 262 hours, but showed less signs of distress throughout their growth, and produced 1·913 gm. dry matter as against 1·055 gm.[1] In the summer, however, the greenhouse was shaded and the leaves did not receive the full force of the sun's rays, the harmful action of excessive insolation being thus mitigated, enabling the plants to make better growth than when they were exposed to the full power of the sun, although acting over a much shorter total period in the latter case. It would appear, therefore, that a high degree of insolation (excessive power of the sun's rays) is a more potent factor for harm than either high temperature or the actual total duration of sunshine.

---

[1] For fair comparison only those plants growing in the same situation under the sloping roof are here taken into consideration though it happens that for May–June the mean of these is the same as that of the whole series (p. 199).

Further experiments were undertaken to ascertain whether the harmful effects of excessive insolation could be reduced by alteration in temperature conditions. As has been already indicated, the difference between the day and night temperatures of water culture solutions is often considerable, especially in hot weather, when it may be 22·5° C. on occasion. This is considerably greater than the fluctuation occurring under soil conditions in the open, where the minimum soil temperature remains considerably above the air minimum, especially in the summer (5), and the maximum does not rise so high as in the water culture solution under glass. In dull weather the maximum and minimum temperatures approximate more closely, as there is less heating up during the day and a less marked fall in the temperature of the glasshouse at night. A method was therefore devised whereby the plants were subjected to a more even temperature at the roots, in order to ascertain whether this affected growth to any appreciable extent at different seasons of the year. The whole of the practical work in connection with this experiment was carried through by Professor Kharak Singh, of Lyallpur, India.

Two 100 gallon tanks were set up, with an outlet pipe from below the rim running down inside to within an inch of the bottom of the tank. Water was admitted from above at the other end of the tank and kept running day and night, so that a continuous slow circulation was maintained. A platform weighted with bricks to carry the water culture bottles was so arranged as to bring the necks to the rim of the tank, just above the constant level of the water. To exclude the light from the roots black cotton covers were fastened round each bottle, as the ordinary paper coats are useless when submerged, and the necks were painted with black enamel in addition. A platform of similar height and size was placed close by to carry a set of bottles in which the variation of temperature was not controlled by a water jacket, both tanks and table being under the sloping roof of the glasshouse. Under these conditions the shoots of the peas were subjected to similar insolation and air temperature, but the temperature at the roots varied with the situation. Twenty-four plants were grown in each case, spaced far enough apart to avoid any overshadowing. Maximum and minimum thermometers were placed in several of the bottles and readings taken daily, and the nutrient solutions were changed frequently. Two experiments were carried through:

(1) In spring, during the most favourable period for growth under greenhouse conditions;

(2) In summer, during the time that premature death of the plants usually occurs.

(1) SPRING EXPERIMENT.

Sutton's Harbinger Peas—April 18th to June 16th.

Growth proceeded satisfactorily with all the plants, and for some time little difference was manifest; eventually the plants on the table began to draw slightly ahead of those in the tanks, and they came into flower somewhat earlier. When cut most of the plants showed incipient signs of dying, as the upper leaves were turning yellow, indicating completion of growth, but comparatively little difference was noticeable between the two sets. The mean dry weights proved to be

	Shoot gm.	Root gm.	Total gm.	Ratio shoot/root
On table	4·284 ± ·109	·885 ± ·028	5·169 ± ·132	5·121 ± ·168
On tank	3·808 ± ·055	·753 ± ·020	4·561 ± ·056	5·215 ± ·143

The mean weekly temperatures (Fig. 1) show a difference of about 8–11° C. between the maximum of table and tank, and 3–5·5° C. between the minima. In all cases the tank maxima were below those of the table, and the minima above, as the surrounding water prevented extreme fluctuations in either direction. On the table the mean maxima ranged 15·5–22° C. above the minima, whereas in the tank the difference was only 3–5° C. Nevertheless, in spite of these considerable differences in root temperature, both as regards the actual temperature reached and the daily fluctuations between maximum and minimum, the growth of the plants was much less affected than might have been anticipated, those on the table being somewhat the heavier. The improvement in the latter case may be attributable to the higher average mean temperatures prevailing throughout the experiment, while it was also probably influenced by the rather low temperatures at the beginning, when the warmer conditions on the table gave the plants the advantage of a better start by enabling them to grow more rapidly at first. This early start was very important, as by the working of the compound interest law it gave these plants a lead which those in the tank were never able to overtake. The ratio of shoot to root was the same in each set, within experimental

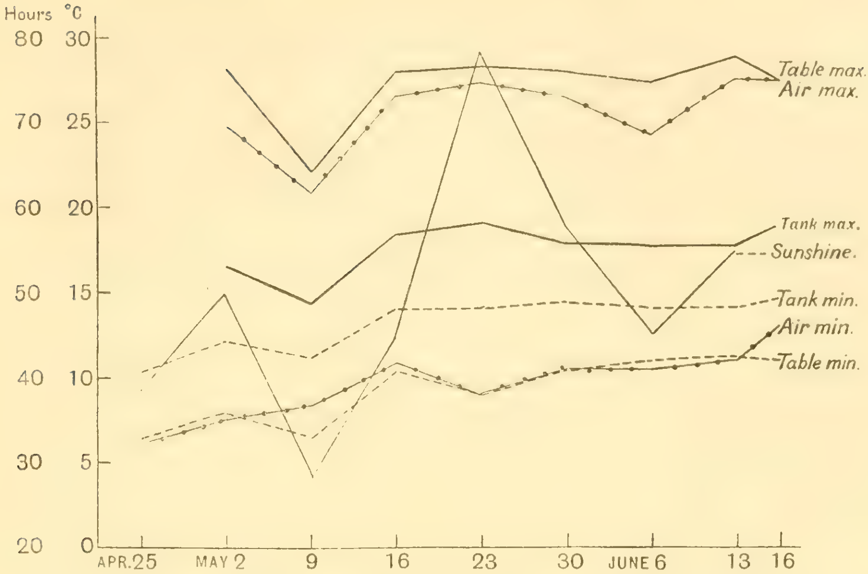


Fig. 1. Temperature records and hours of sunshine, April 19th–June 16th, 1921.

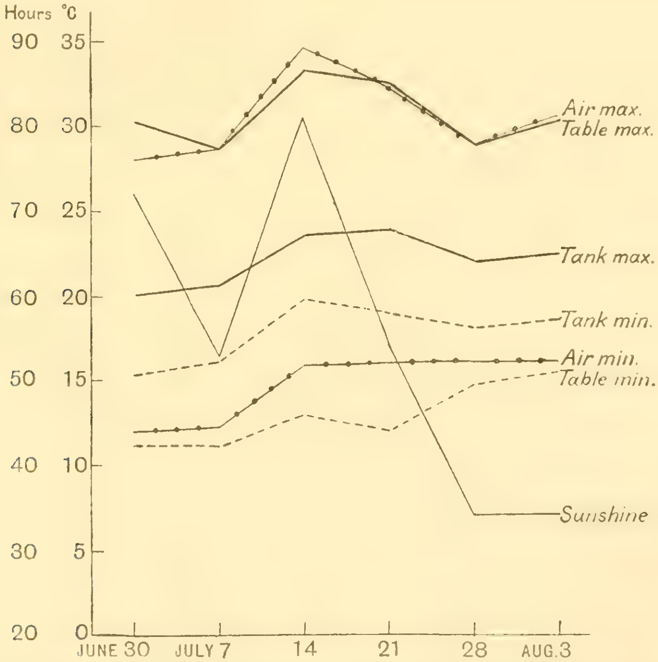


Fig. 2. Temperature records and hours of sunshine, June 24th–Aug. 3rd, 1921.

error, showing that the variable temperature had not caused any change in the development of the roots compared with that of the shoots.

The daily average of sunshine over the whole period was seven hours. During the first month the total hours per week were somewhat low, but May 16th–23rd was a very sunny week, ten to fourteen hours being registered on each of five days. After this no further period of excessive sunshine was recorded. At first the temperatures fluctuated to some degree with the amount of sunshine, but later were independent of it, for when the total sunshine dropped during the last five weeks, the mean temperature remained very constant and high, 27–28·5° C.

It would thus appear that under similar conditions of light and provided no inhibiting factor such as excessive insolation comes into play, the amount of daily fluctuation of root temperature has comparatively little effect on the growth of peas within a total mean range of 7–29° C., provided that the mean temperatures do not vary considerably. These are the limits in the experiment under consideration and possibly might be extended to some degree in either direction. Within those limits a large variation in maxima, up to 11° C., will permit of much the same amount of growth as measured by dry weight, though a low mean maximum (below 16° C.) in the early stages may cause some retardation. Growth proceeds equally well whether the temperatures at the roots are fairly even, varying within 5° C., or whether they fluctuate as much as 22° C., on the average, *i.e.* within certain limits high maximum temperatures associated with low minima have the same ultimate effect on growth as low maxima and high minima.

(2) SUMMER EXPERIMENT.

Sutton's Harbinger Peas—June 24th to August 3rd.

The experiment was begun in hot sunny weather when temperatures ruled very high and the number of hours of sunshine was excessive. Very soon many of the unprotected plants on the table began to show signs of distress, turning pale and wilting, and within eighteen days many were dead. In six weeks there were only four survivors, and these were small and distinctly unhappy. The plants in the tank grew well from the beginning and remained green and healthy to the end, only one failing. At the time of cutting the upper leaves were just beginning to turn yellow, showing growth was finished. The mean dry weights were:

	Shoot gm.	Root gm.	Total gm.	Ratio shoot/root
Table (4 plants only)	1·157 ± ·091	·214 ± ·026	1·371 ± ·109	5·63 ± ·478
Tank (23 plants)	1·839 ± ·007	·227 ± ·075	2·066 ± ·078	8·26 ± ·289

The plants in which the roots were protected from excessively high temperatures made therefore about half as much growth again as the unprotected survivors on the table. The increase was chiefly due to shoot growth as the roots weighed much the same in both cases, thus suggesting that the injurious action of combined strong insolation and high temperature is more marked on the assimilatory tissues than on the roots, the organs of absorption, the ratio of shoot to root being thus reduced. This is in contrast to what happens when growth is adversely affected by overcrowding, in which case the shoot/root ratio increases(3), probably owing to an attempt on the part of the plant to increase its assimilatory surface in view of the decreased illumination.

Throughout the period the mean temperatures in the solutions were from 4–5·5° C. higher than during the earlier test, all being above the highest means previously registered, but the differences between the table and tank maxima and minima were very much the same in both cases. The table maxima, however, ruled very high, ranging from 28·6–33·3° C., *i.e.* at temperatures above the initial optimum, which would cause a depression in the rate of growth during their period of operation. Added to this, there was a great deal of strong sunshine during the first and third weeks, and this association of excessive insolation with high root temperatures wrought havoc among the plants on the table, and gave them a very bad start. During the last three weeks there was a great drop in the amount of sunshine, but the temperatures remained high, so that at the end of the period the temperature effect was the more marked. The same amount of sunshine, however, had far less detrimental effect when the roots were kept cooler, and not only did nearly all the plants in the tanks survive, but they made much greater individual growth.

Nevertheless, a comparison of the dry weights shows that the conditions in the later test were less favourable even in the tank, though the depreciation was not nearly so great as on the table.

Total dry weights.

	April—June gm.	July—Aug. gm.
Tank	4·561	2·066
Table	5·169	1·371

Growth in the second experiment was practically finished in six weeks instead of in eight, but with the speeding up less than half as much dry weight was produced. This may possibly be attributed to the excessive insolation rather than to the high root temperatures, as the tank maxima were much lower than the table maxima of the spring experiment and so were under the limits at which growth is adversely affected. On the other hand, the mean minima ranged several degrees (4–8·5° C.) higher than in the earlier test. Previous experiments with peas (3) have shown that with high maximum temperatures a rise in minima is disadvantageous and checks growth considerably. Temperatures of 13–15·5° C. are distinctly harmful when associated with 26·5–35° C. as maxima. In the present case the mean minima were higher and ranged from 15·5–20° C., being above 18·5° C. for most of the time, and may have exercised a harmful effect even though the associated mean maxima only reached 20–24° C. The total growth in the tank in summer may therefore have been depressed by the high minimum temperatures as well as by the excessive insolation, but the influence of these two factors cannot yet be dissociated.

SUMMARY.

1. Under ordinary environmental conditions of temperature and sunlight the growth of peas, as of barley, is seriously hindered by overcrowding, even when each plant receives a similar supply of food and water. Not only is less dry weight produced, but the pods become thin and distorted and fail to develop their seeds properly.

2. Growth tends to be depressed in hot sunny weather when no protection is afforded. The chief detrimental factors concerned appear to be high temperatures at the roots associated with strong and prolonged sunshine, though the two factors acting individually are much less potent for harm. Under these conditions crowding shelters the roots from overheating and the leaves from too much sunlight, and up to a certain point crowded plants make better growth than those spaced well apart. Overcrowding, however, still depresses growth, probably because the light and root temperature reductions are too great.

3. Provided insolation is not excessive the amount of daily fluctuation of root temperature over a total range of about 22° C. (6·7–28·9° C.) has comparatively little influence upon growth; high maxima and low minima give similar results to low maxima and relatively high minima, provided the average mean temperatures are not too dissimilar.

4. With high root temperatures a difference in the degree of insolation or in the angle of incidence of the sun's rays may have a considerable influence on growth, a slight easing off of the solar conditions enabling much better growth to be made.

5. With very strong sunshine reduction of high maximum root temperatures (from 29° C. upwards) allows of satisfactory growth, when unprotected plants are rapidly killed. The inhibitory action of too high temperatures at the roots is thus clearly shown.

Nevertheless, the growth so made is less good than under more normal conditions of insolation, thus demonstrating the harmful action of too powerful sunlight, when all the root temperatures rule high.

6. Root temperatures appear to be of greater importance than atmospheric temperatures, as good growth can be made in hot atmospheres provided the roots are kept relatively cool.

7. There is some reason to believe that the minima are of as much importance as the maxima, *i.e.* that plants can withstand very high maximum temperatures provided there is a considerable drop to the minima, but cannot put up with the constant conditions of heat induced by fairly high maxima and high minima.

REFERENCES.

(1) ASKENASY, E. (1890). Ueber einige Beziehungen zwischen Wachstum und Temperatur. *Ber. d. Deut. Bot. Gesells.* VIII. 61–94.

(2) BALLS, L. (1908). Temperature and Growth. *Ann. Bot.* XXII. 557–591.

(3) BRENCHLEY, W. E. (1919). Some factors in plant competition. *Ann. App. Biol.* VI. 142–170.

(4) —— (1920). On the relations between growth and the environmental conditions of temperature and bright sunshine. *Ann. App. Biol.* VII. 211–244.

(5) KEEN, B. A. and RUSSELL, E. J. (1921). The factors determining soil temperature. *Journ. Agric. Sci.* XI. 211–239.

(6) LEHENBAUER, P. A. (1914). Growth of Maize seedlings in relation to temperature. *Physiol. Res.* I. 247–288.

(7) LEITCH, I. (1916). Some experiments on the influence of temperature on the rate of growth in *Pisum sativum*. *Ann. Bot.* XXX. 25–46.

(*Received June* 10*th*, 1922.)

STUDIES IN BACTERIOSIS. VII

COMPARISON OF THE "STRIPE DISEASE" WITH THE "GRAND RAPIDS DISEASE" OF TOMATO

BY SYDNEY G. PAINE AND MARGARET S. LACEY.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

IN the course of an investigation of the "Stripe Disease" of tomatoes a yellow organism was frequently found associated with the causal organism. The properties of this organism were so similar to those of *Aplanobacter michiganense*, E.F.S. (1) as to suggest close relationship with it, and in a former communication (2) the question was raised whether on further investigation the Grand Rapids Disease might not prove to have a common etiology with the "Stripe Disease."

By the courtesy of Dr E. F. Smith and Professor Nakata, to both of whom the authors wish to express their thanks, a tomato plant infected with the Grand Rapids Disease was obtained and a careful comparison of the two organisms rendered possible. From this plant *Aplanobacter michiganense* was isolated without difficulty and compared with the yellow organism referred to above. The two organisms were given preliminary culture by several transfers in broth tubes and their characteristics on different media were then investigated with the following result.

	Aplanobacter (Paine and Bewley)	*Aplanobacter michiganense* E.F.S.
Pathogenicity	Not pathogenic for tomato when associated with *B. lathyri*	Pathogenic for tomato
Bouillon-agar plate culture	Colonies round, smooth, glistening, viscous, deep orange	Colonies round, smooth, glistening, very pale yellow at first deepening with age to a mid-chrome
Bouillon-agar slope ...	Deep orange, very viscous	Pale yellow, not nearly so viscous
Broth	Slow growth, clear after 24 hours, cloudy after 48 hours, no pellicle	The same
Gelatine slope	Liquefaction starts in 48 hours	No liquefaction until the 11th day
Nitrate broth	No reduction of nitrate	The same

	Aplanobacter (Paine and Bewley)	*Aplanobacter michiganense* E.F.S.
Glucose broth	Slight acid on 6th day litmus reduced, pellicle	Slight acid on 13th day litmus not reduced, no pellicle
Lactose broth	Slight acid on 9th day litmus reduced, pellicle	Slight acid on 13th day litmus not reduced, no pellicle
Sucrose broth	Slight acid on 6th day litmus reduced, pellicle	Slight acid on 13th day litmus not reduced, no pellicle
Potato plug	Thick viscous growth, wet shining, deep orange, potato dark grey	Growth not nearly so viscous, pale yellow, potato pinkish
Diastatic action	Strong	Strong
Milk tubes...	No clotting, digestion of casein apparent after 9 days, complete in 3 weeks	No clotting, casein digestion not apparent until the 14th day, only slight after 30 days
Indol formation	Slight sign	No sign
Staining	Gram positive when first isolated but later became gram negative	Gram positive
Size and shape	Small oval rods, $1{\cdot}6\mu \times 0{\cdot}6\mu$	Small oval rods, $1{\cdot}6\mu \times 0{\cdot}6\mu$
Motility	Non-motile	Non-motile

From the above it is seen that while these two organisms possess many properties in common, certain differences, mainly differences in degree only, clearly mark them as different species and the name *Aplanobacter dissimulans* is now proposed for the species isolated by Paine and Bewley.

INFECTION EXPERIMENTS.

Inoculations of young tomato plants were carried out in the experimental houses at Waltham Cross by Dr W. F. Bewley, to whom the authors tender their best thanks. The two *Aplanobacters* and *Bacillus lathyri* were pricked separately into three sets of eighteen plants; the results with *Aplanobacter dissimulans* were negative in every case; many successful infections were obtained with *B. lathyri* and *A. michiganense*, the effect upon the pith was identical and altogether indistinguishable, but marked differences were observed when the organisms, having passed through the cortex, produced lesions on the exterior of the stem. The former produced dark brown sunken furrows with usually no cracking of the epidermis, while the latter gave no special colouring but produced deep fissures in the outer cortex whose margins had the appearance of callus formations; no effect upon the fruits was observed in the case of *Aplanobacter michiganense*. The two diseases therefore appear to be entirely distinct, and the senior author desires to withdraw the suggestion made in the former paper, to which reference has been made above, that in his investigation of the Grand Rapids Disease Dr Smith had possibly been in error as to the etiology of the disease.

SUMMARY.

1. The Stripe Disease and the Grand Rapids Disease of tomato are distinct diseases caused by two bacterial parasites, *Bacillus lathyri* and *Aplanobacter michiganense*.

2. The yellow organism, *Aplanobacter dissimulans* n.sp. (Paine and Bewley), which is frequently found associated with *Bacillus lathyri* is not identical with *Aplanobacter michiganense*.

REFERENCES.

(1) SMITH, E. F. (1914). The "Grand Rapids Disease" of Tomato. *Bacteria in relation to Plant Diseases*, Washington, D.C. III. 161.

(2) PAINE, S. G. and BEWLEY, W. F. (1919). Studies in Bacteriosis. IV. "Stripe Disease" of Tomato. *Ann. App. Biol.* VI. 183.

(*Received June 26th*, 1922.)

THE INFESTATION OF FUNGUS CULTURES BY MITES[1]

(ITS NATURE AND CONTROL TOGETHER WITH SOME REMARKS ON THE TOXIC PROPERTIES OF PYRIDINE)

By SIBYL T. JEWSON, M.Sc.
(*Department of Mycology, Rothamsted Experimental Station, Harpenden*).
AND F. TATTERSFIELD, B.Sc., F.I.C.
(*Rothamsted Experimental Station*).

(With 4 Text-figures.)

CONTENTS.

		PAGE
1.	Introduction	213
2.	Nature of Infestation	214
3.	The Problem of Control	215
4.	Experimental	215
5.	Quality of Pyridine	217
6.	Toxicity of Pyridine to the eggs of Mites	219
7.	Action of Pyridine on Mites (Quantitative)	222
8.	Toxic action of Pyridine on Fungi	229
9.	Discussion of Results	237
10.	Application of Method	239
11.	Summary and Conclusions	239

1. INTRODUCTION.

THE infestation of pure cultures of fungi by mites is a considerable source of trouble in mycological laboratories. This difficulty having arisen at Rothamsted, it was considered advisable to make a careful study of the nature of the infestation and the toxic effect of a number of volatile organic chemical compounds on these pests and on fungi. The object of the investigation was to find a method of controlling the mite infection without injuring the fungi. Once having gained access to the laboratory

---

[1] A grant in aid of publication has been received for this communication.

mites make their way through cotton-wool plugs of culture tubes. Besides destroying the culture they have entered, they may make accurate subculturing a matter of difficulty by reason of the extraneous matter—bacteria, fungus spores, etc.—they carry with them into the tube. They wander rapidly from tube to tube and, unless discovered at an early stage, the whole set of cultures in a laboratory may be either destroyed or seriously contaminated. Even if the cultures be abandoned and a completely fresh start made, another infestation may readily take place from eggs laid in some unnoticed corner of the laboratory.

2. NATURE OF THE INFESTATION.

Three species of mites were found contaminating cultures of which *Aleurobius farinae*, De Geer, the Flour Mite, was the most abundant and widespread. In many cases infection was slight; in others eggs, larvae and adults were present but the mycelium was not noticeably destroyed by this species. *Tyroglyphus longior*, Gervais, one of the cheese mites, was observed in a few cultures. In most cases infection by this species was very slight, but in three cultures of a species of the fungus *Trichoderma* the whole of the fungus was destroyed and the medium was blackened with faecal pellets. *Glyciphagus cadaverum*, Schrank, was found only in one set of cultures. The eggs of the two latter species were not recorded. These three species are among those termed "Forage Mites" as distinguished from "Mange Mites." They infect many kinds of grain and flour and can frequently be found in the dust from crevices in houses or stables. *T. longior* and *A. farinae* are also two of the species that attack Stilton and Cheddar cheeses. The life histories of all three species are very similar, consisting of four stages, egg, larva, nymph and adult. That of *T. longior* has been described by Eales(1). The life cycle is completed in four to five weeks, the eggs hatching about 10–12 days after being laid. The larva is distinguished from the later stages by having only three pairs of legs. It feeds actively for about a week, then becomes quiescent and casts its skin, emerging as the first nymph. This moults and becomes the second nymph which after a third moult emerges as an adult male or female. There may, under favourable conditions, be an additional hypopial stage, the hypopus being specially adapted for distribution. It has a resistant skin and on the ventral surface there is a sucker by which it can attach itself to flies, moths or human beings. The life cycle of *A. farinae* as described by Newstead and Duvall(2) is very similar but usually shorter, varying from about 17 days in July to 28 days in the winter months. The eggs usually hatch in about 3–4 days. There is only one nymphal stage and the hypopus is very rare. *G. cadaverum* has a similar life history but the details are not well known.

3. The Problem of Control.

The *sine qua non* of any method of control is that the treatment should kill 100 per cent. of the mites and their eggs and have a minimum detrimental effect upon the fungus cultures. It should not be harmful to the operator and it should be easy to apply. If a chemical method is to be used it is essential that the substance be volatile, not too disagreeable, and that in its toxic action it should be reasonably speedy. In flour mills it is customary to keep mites under control by scrupulous cleanliness and where necessary by the application of heat. The lowest lethal temperature was found by Newstead and Duvall(2) to be 49° C. applied for at least 12 hours. This latter method was not available in our case as the temperatures likely to be effective against the parasite would have a seriously detrimental action upon the fungus culture. A fairly extensive list of volatile organic compounds was therefore tried and their effect studied upon mites and their eggs and upon fungi.

Ammonia was found to be the most rapidly toxic substance to mites and their eggs. It had, however, a definite toxic action on fungi and although it may prove of great value for ridding laboratory apparatus, such as incubators, of these pests, its vapour should not be allowed to play upon the cultures of fungi for any length of time.

Pyridine was the next most rapidly toxic compound tested and although it is many times less toxic than ammonia vapour, it has the added advantage of not being poisonous to fungi, except in doses not likely to occur in practice. As its vapour is rather disagreeable it is hardly suitable for the purposes for which ammonia is recommended, but for freeing fungus cultures of mite pests it can be so easily applied that it should not prove in any way obnoxious to the operator. A detailed description of both methods is given on p. 239.

4. Experimental.

The compounds tested were:

Ammonia bases	Ammonia Pyridine Aniline Monomethylaniline Dimethylaniline
Aromatic hydrocarbons	Benzene Toluene Naphthalene
	Para-dichlorbenzene Carbon tetrachloride Carbon bisulphide

All these compounds were chosen because of their definite insecticidal value. With the exception of Ammonia, Mono- and Dimethylaniline this is not considerable but it was thought to be sufficiently high for the substances to prove effective in air saturated with their vapour against a not very resistant pest. Moreover, it was considered that the toxic effect of the majority of them to fungi would be small.

(*a*) *Action upon Fungi.*

As it was essential that the latter condition should be complied with, these substances were all given a preliminary test to discover their action against a common fungus. A green *Penicillium* was used, the cultures being tested in duplicate, one of each couple being exposed with the cotton-wool plug *in situ*, the other with it removed[1]. The culture tubes were put into a large boiling tube containing a quantity of the chemical, sufficient to saturate the air with its vapour. The boiling tube was then corked and put aside for three days after which the culture was taken out and subcultured. The results are stated in Table I.

Table I.

Effect of Vapour of various Organic Chemicals on Penicillium *sp. Culture exposed for three days and then subcultured.*

	Chemical	Growth of subculture after 7 days
1.	Ammonia	No growth
2.	Pyridine	Good growth
3.	Aniline	,,
4.	Monomethylaniline	,,
5.	Dimethylaniline	,,
6.	Benzene	,,
7.	Toluene	Fairly good growth
8.	Naphthalene	Good growth
9.	*p*-Dichlorbenzene	Good growth
10.	Carbon tetrachloride	One fair growth and one slight growth
11.	Carbon bisulphide	No growth

(*b*) *Action upon Mites.*

A selection of the above compounds was then tested upon mites. Some flour mites, *A. farinae*, were placed in tubes which were vaselined round the outer lip to prevent the escape of the mites, but left unplugged. Exposure to the toxic substance was made in exactly the same way as described but for varying lengths of time. The results are shown in Table II.

---

[1] The results of the two series showed no significant differences.

Table II.

Action of the Vapour of Certain Organic Chemical Compounds on Mites (Aleurobius farinae).

Chemical	Action after 3–4 hours	Action after 16 hours
Pyridine (1) Pure ...	—	All appeared dead.
,, (2) ...	After 4 hours all mites appeared dead. Some of larger ones recovered a day later and some eggs hatched out.	—
,, (3) Commer'l.	—	All appeared dead.
Aniline	—	Many appeared dead but most of the large and some small ones moved sluggishly.
Monomethylaniline	—	A few of both large and small ones alive but sluggish.
Dimethylaniline	—	Large mites alive but sluggish.
Naphthalene ...	No apparent effect.	No apparent effect.
p-Dichlorbenzene ...	All anaesthetised.	All apparently anaesthetised but some recovered on exposure to air.
Carbon bisulphide	All apparently dead.	All apparently dead.

An inspection of Tables I and II clearly indicates that for practical purposes Pyridine is much the most hopeful compound. Carbon bisulphide, although apparently rapid in its action, is too toxic to fungi to be useful, while Paradichlorbenzene, which, from its slight poisonous action on fungi and from the almost complete absence of disagreeable properties, would have been an ideal substance to apply, seems to have only a pronounced but temporary anaesthetic effect.

In view of these results it was decided to make a more complete study of the toxic action of Pyridine and to ascertain, if possible in a quantitative way, its reaction with both mites and some common fungus.

5. THE QUALITY OF THE PYRIDINE USED IN THE EXPERIMENT.

Four samples of Pyridine were tested for their effect upon mites and fungi.

1. A sample labelled pure Pyridine.
2. A sample obtained by a rough fractionation of commercial Pyridine.
3. Commercial Pyridine.
4. A sample carefully purified in the Laboratory.

No. 2 sample was intermediate in quality between samples 1 and 3.

The testing of these grades was regarded as necessary owing to the wide discrepancy in price between pure and commercial Pyridine. It was also essential to ascertain whether through the presence of any impurity commercial Pyridine would prove deleterious to fungi and so inhibit its use or render its fractionation and purification indispensable. Moreover, it was important to ascertain whether the actual toxic product in the commercial article was Pyridine itself or some impurity.

For purposes of reference and comparison the specific gravity and the fractions distilling at various temperatures were determined.

The distillations were carried out in the following way:

75 c.c. were distilled at a rate of one drop per second from a 150 c.c. flask (neck 9 cm. long, diameter of bulb 6·5 cm.) fitted with a four-pear fractionating column of a length from bottom to side tube of 24·5 cm.

The column was so adjusted into the neck of the flask that the total length of still-head was just about 30 cm. A Davies double jacketed condenser in a perpendicular position was attached to the side tube of the column. The distillates were collected and measured. The results are tabulated in Table III.

Table III.

Fractional Distillation of Three Samples of Pyridine.
75 *c.c. distilled at a rate of* 1 *drop per second.*

Sample of Pyridine labelled Pure. S.G. at 15·5° C., 1·0098				Crude Pyridine fraction from Commercial Pyridine. S.G. at 15·5° C., 1·0092				Commercial Pyridine. S.G. at 15·5° C., 0·99307			
No.	Temp. ° C.	Vol. of distillate	% of distillate	No.	Temp. ° C.	Vol. of distillate	% of distillate	No.	Temp. ° C.	Vol. of distillate	% of distillate
	92	1st drop			88	1st drop			94	1st drop	
1.	92–110	0·2 c.c.	·27	1.	88–110	3·5 c.c	4·67	1 *a.*	94–100	30 c.c.	40·0
								1 *b.*	100–110	5·3	7·07
2 *a.*	110–115	1·3	1·73	2.	110–120	13·5	18·00	2 *a.*	110–115	2·0	2·67
2 *b.*	115–120	25·0	33·34					2 *b.*	115–120	4·0	5·33
3 *a.*	120–125	30·0	40·0	3 *a.*	120–125	29·5	39·33	3 *a.*	120–125	16·3	21·73
3 *b.*	125–130	8·0	10·67	3 *b.*	125–130	10·5	14·00	3 *b.*	125–130	6·9	9·2
4.	130–140	7·7	10·26	4.	130–140	9·0	12·0	4.	130–140	7·0	9·33
5.	Residue	2·8	3·73	5.	140–150	6·5	8·67	5.	Residue	3·5	4·67
					Residue	2·5	3·33				

An inspection of this table shows that there are wide differences in the composition of the three samples, that the commercial product (which contains about 34 per cent. water) has a considerable fraction distilling off between 95° and 100° C., and that the sample labelled Pure is misrepresented. The last point was confirmed by testing with Permanganate which was rapidly decolourised. In view of the obvious impurity of the Pyridine labelled "Pure," this sample was treated with Potassium Permanganate, dried over solid Caustic Potash and fractionated. The fraction distilling between 115° and 125° C. was again treated with Permanganate and again fractionated. The fraction distilling between 114° and 117° C. was collected and tested quantitatively for its toxicity to mites. The pure and commercial Pyridine were tested for their toxic action to mites and their eggs.

Two samples of cheese mites were obtained and identified as *T. longior*, or a species very closely related to it. Both samples contained a large number of eggs. Duplicate tubes of the mites were then treated in bell-jars with (*a*) Pyridine (1 above), and (*b*) commercial Pyridine (3 above) for a period of 16 hours, two controls being set aside over water for purposes of comparison. At the end of this period they were examined and in all the treated samples the mites showed no signs of life.

After a period of fourteen days all the samples were re-examined with the result that whereas one of the controls showed many young and lively mites and comparatively few unhatched eggs and the other a few large live mites and a large number of unhatched eggs, the tubes exposed to the vapour of both samples of Pyridine contained no live mites, either adults or newly hatched larvae. This and many subsequent experiments amply proved that there is little or no difference in toxic action between the costly pure Pyridine and the cheap commercial article.

6. TOXICITY OF PYRIDINE TO THE EGGS OF MITES.

The critical point in the method is the toxicity of Pyridine to the eggs of mites, for unless all are killed the infection is not eliminated. This matter was therefore studied with considerable care, the actual experiments being repeated several times to eliminate chance results due to such factors as the Pyridine not penetrating a thick mass of mites or to the sample undergoing desiccation during the aeration subsequent to the experiment.

The results in one case (Series I) do not agree with those obtained at any other time, but they are set out in Table IV*a* with the purpose of indicating that a sixteen-hour exposure which we have generally found to be ample to kill all mites and eggs may fail in certain cases and as a consequence we suggest that with very heavy infestations a second exposure may be necessary after a period of fourteen days.

Series I was exposed in duplicate for 16 hours to vapour of three qualities of Pyridine. A little flour was placed in each tube to provide a food supply for any larvae hatching out. After treatment both mites and flour were transferred to fresh tubes and the excess of Pyridine allowed to escape. The result was definitely negative and might be due either to the cold weather prevailing at the time diminishing the concentration of Pyridine in the air of the bell-jar, or to the eggs being rather more resistant in this case or under these conditions.

Series II was exposed to the vapour of commercial Pyridine for two different periods, 16 and 48 hours, in each case in duplicate. The samples were then transferred to Petri dishes and exposed to the moist air of a warm greenhouse for eight hours to free the material from traces of Pyridine as completely and rapidly as possible. The samples were then transferred back to tubes and allowed to stand in a damp atmosphere for 16 to 19 days, small portions being examined from time to time.

The results of both series are shown in Table IV*a*.

Table IV*a*.

Showing effect of Pyridine upon Mites and Eggs.
(*Sample contained about equal numbers of each.*)

Series I.

Exposure to Vapour of Pyridine for 16 *hours.*

Set on 18/1/22.

Examined	Days after taking off	Controls	Pure Pyridine (1)	Commercial Pyridine fractionated (2)	Commercial Pyridine (3)
19/1/22	—	Active—all stages	Apparently dead unhatched eggs	Apparently dead unhatched eggs	Apparently dead unhatched eggs
23/1/22	4	,, ,,	One mature live mite	Apparently dead unhatched eggs	Apparently dead unhatched eggs
27/1/22	8	,, ,,	One tube app. dead; second tube live larvae	Live larvae (both tubes)	Apparently dead unhatched eggs
31/1/22	12	,, ,,	One adult, one larva; unhatched eggs	Many larvae; unhatched eggs	One live larva; unhatched eggs
7/2/22	19	,, ,,	Eggs, nymphs and adults	Eggs, nymphs and adults	Eggs, nymphs and adults

Series II.

Exposure to Vapour of Pyridine for 16 *and* 48 *hours.*

Set on 20 and 21/2/22.

Examined	Days after taking off	Controls	Commercial Pyridine	
			Exposure 16 hrs.	Exposure 48 hrs.
22/2/22	—	Active—all stages	All apparently dead	All apparently dead
27/2/22	5	,, ,,	No live mites	No live mites
28/2/22	6	,, ,,	,, ,,	,, ,,
3/3/22	9	,, ,,	,, ,,	,, ,,
6/3/22	12	,, ,,	,, ,,	,, ,,
10/2/22	16	,, ,,	,, ,,	,, ,,

The eggs are obviously much more resistant than the mites themselves and it is apparent from the discrepancy between Series I and II that a slight change in the conditions of carrying out the experiment may lead to failure. In view of this a fresh series of experiments (III) was set up, the results being shown in Table IV*b*, which also gives the action of Ammonia vapour on the eggs.

Table IV*b*.

Series III. *Effect of Pyridine and Ammonia on Eggs of Cheese-mites.*

Temp. of exposure 18°–19° C.
Exposure in flasks sealed with lead-lined stoppers.
Air of flask saturated with vapour.
Time elapsing before examination is reckoned from time of taking out of flasks.

Pyridine.

No.	Description	Duration of exposure	Exam'd after	Mites alive	Eggs not hatched	Exam'd after	Mites alive	Eggs not hatched	Exam'd after	Mites alive	Eggs not hatched
1.	Control (*a*) (dry flask)	72 hrs.	14 days	Many	Some	18 days	Many	Some	25 days	Many	Few
2.	Control (*b*) (damp flask)	72 ,,	14 ,,	,,	Some	18 ,,	,,	Some	25 ,,	,,	,,
3.	Pyridine (pure)	3 ,,	17 ,,	,,	V. few	21 ,,	,,	V. few	28 ,,	,,	,,
4.		8 ,,	17 ,,	,,	Many	21 ,,	,,	Many	28 ,,	,,	Many
5.		16 ,,	16 ,,	None	,,	20 ,,	None	,,	27 ,,	None	,,
6.		24 ,,	16 ,,	,,	,,	20 ,,	,,	,,	27 ,,	,,	,,
7.		36 ,,	—	—	—	19 ,,	,,	,,	26 ,,	,,	,,
8.		48 ,,	—	—	—	19 ,,	,,	,,	26 ,,	,,	,,
9.		72 ,,	—	—	—	18 ,,	,,	,,	25 ,,	,,	,,
10.	Pyridine (commer'l)	3 ,,	17 ,,	Many	V. few	21 ,,	Many	V. few	28 ,,	Many	Few
11.		8 ,,	17 ,,	,,	Many	21 ,,	,,	Many	28 ,,	,,	Many
12.		16 ,,	16 ,,	None	,,	20 ,,	None	,,	27 ,,	None	,,
13.		24 ,,	16 ,,	,,	,,	20 ,,	,,	,,	27 ,,	,,	,,
14.		36 ,,	—	—	—	19 ,,	,,	,,	26 ,,	,,	,,
15.		48 ,,	—	—	—	19 ,,	,,	,,	26 ,,	,,	,,
16.		72 ,,	—	—	—	18 ,,	,,	,,	25 ,,	,,	,,

Ammonia.

Series I.

No.	Description	Duration of Exposure	Examined after	Mites alive	Eggs not hatched	Examined after	Mites alive	Eggs not hatched
1.	Control	—	10 days	Many	Few	—	—	—
2.	,,	—	,,	,,	,,	—	—	—
3.	Ammonia	2 mins.	,,	,,	,,	—	—	—
4.	,,	5 ,,	,,	,,	,,	—	—	—
5.	,,	15 ,,	,,	None	Many	17 days	A few	Many
6.	,,	30 ,,	,,	A few	,,	,,	,,	,,

Series II.

No.	Description	Duration of Exposure	Examined after	Mites alive	Eggs not hatched	Examined after	Mites alive	Eggs not hatched
1.	Control	—	20 days	Many	—	23 days	Many	—
2.	Ammonia	15 mins.	,,	A few	Many	,,	Some	Some
3.	,,	30 ,,	,,	None	,,	,,	None	,,
4.	,,	60 ,,	,,	,,	,,	,,	,,	Many
5.	,,	120 ,,	,,	,,	Some	,,	,,	,,

In Series III the lethal chambers were conical flasks of about 1200 c.c. capacity. Small samples containing many eggs and a few mites (100 to 28) were placed in small tubes, the mouths of which were covered with fine silk gauze, and suspended in the vapour in the flask at a temperature of 18°–19° C. for varying lengths of time. The flasks were hermetically sealed by lead-lined rubber stoppers. After exposure the samples were poured out into small flat dishes and exposed to the atmosphere for 15 to 30 minutes until the odour of Pyridine had almost disappeared. They were then placed in a large bell-jar containing a basin of water and left overnight, after which they were transferred to tubes with the addition of a little flour and kept at 18°–19° C. in a moist atmosphere.

Two samples of Pyridine, one practically chemically pure, the other labelled commercial, were tested in this way for times ranging from three hours, which is just long enough to eliminate the mites, to 72 hours. It is of interest to note that in every case except the controls, the samples became covered with a mat of fungus mycelium, indicating that little danger to fungus growth is to be feared from exposures up to 72 hours. Examination was carried out from time to time up to 28 days. Three hours' exposure was quite ineffective against the eggs, practically all hatching out in 16 days; 8 hours was partially successful as many eggs did not hatch in 20 days, while 16 hours and upwards completely prevented hatching out. No difference whatever could be detected in the lethal properties of the two samples of Pyridine. It is considered that if the treatment be carried out at an equable temperature of about 18°–20° C., 16–24 hours' exposure should be sufficient to eliminate both mites and eggs. It is recognised, however, that there may be cases of heavy infestation when the vapour of Pyridine may not be able to permeate completely and where a second exposure after fourteen days might be advisable before subculturing.

7. ACTION OF PYRIDINE ON MITES (QUANTITATIVE).

An attempt was made to put the results on a quantitative basis. This was deemed advisable because of the rather surprisingly high toxicity of Pyridine in air saturated with its vapour and because materials like Aniline and Dimethylaniline which, from the work of Tattersfield and Roberts (3), were expected by us to have a higher toxic value in the vapour phase than Pyridine had proved of doubtful value. Pyridine, Ammonia and Aniline were therefore compared. For this purpose flasks of about 1100 c.c. capacity were fitted with lead-lined rubber stoppers, through which passed a glass rod turned to a hook at the lower end, to which could be attached a short test tube by means of wire. The first series of experiments was carried out in air saturated with the appropriate vapour. In the case of Pyridine and Aniline a few drops in excess of what was required to saturate the atmosphere were pipetted into the flasks. After a time sufficiently prolonged to allow of the air being saturated, the tubes containing the mites and closed at the top by a little silk fabric of very fine mesh, drawn tight and fastened firmly to prevent the mites from escaping, were inserted by attaching to the hooks and pushing the cork home. With Ammonia 5–10 c.c. of ·880 material was poured in; in this case the toxic action is so rapid as to render the silk fabric unnecessary. Two controls were used for each set of experiments. After varying lengths of time, in the case of Ammonia reckoned in seconds, the tubes were taken out and either examined immediately or after a time.

The method of examination and the time that should elapse before it is carried out were matters of considerable difficulty and need some consideration. It is necessary to count at least a hundred mites to obtain reliable results. Preliminary experiments showed olive oil to be the best medium in which to count the mites under the microscope as they remain alive in it for one to two hours and its clearing action is marked. Those mites which on careful examination showed no sign of movement were regarded as dead. If inspection be carried out immediately after exposure there is a possibility of mistaking temporary anaesthesia or stupefaction for death. Experience showed, however, that this difficulty was not very serious, for the poisons tested appear to act on the motor nerves and a mite once thoroughly incapacitated in this way seems rarely to recover. As a matter of fact, immediate examination gives an under-estimation of the toxic action—but this can hardly be avoided. The dangers of allowing the material to stand overnight appear more serious as even when aerated in open dishes it loses such toxic materials as Pyridine and Aniline only after a little time and at different rates owing to differences in their respective vapour pressures, during which time the poison continues to act. Moreover, in this treatment there is a danger of desiccation, and of some non-poisoned eggs hatching out.

The effect of Pyridine was tested in two ways. The examination in one case was carried out immediately. In the other the treated mites were aerated in the open till the characteristic odour had disappeared; they were then kept for a further sixteen hours in a moist atmosphere, after which they were examined. In the case of Ammonia examination was carried out immediately and after the lapse of an hour or two during

Table V.

Toxicity of Ammonia (NH_4OH), Pyridine and Aniline to Mites. Atmosphere saturated to chemical.

Vapour from Ammonia ·880. Examination immediately after treatment.

					% calculated on live mites in control	
Time of exposure	No. alive	No. dead	% alive	% dead	% alive	% dead
Controls	90	10	90	10	100	—
A. 16 secs.	87	13	87	13	96·6	3·4
B. 32·2 „	78	32	70·9	29·1	78·7	21·3
C. 46·2 „	72	38	65·5	34·5	72·7	27·3
D. 60·5 „	172	138	55·4	44·6	61·5	38·5
E. 75 „	51	306	14·3	85·7	16	84
F. 90 „	44	384	10·2	89·8	11·3	88·7
G. 105 „	8	271	2·8	97·2	3·1	96·9
H. 120·5 „	10	263	3·6	96·4	4	96

Vapour of pure Pyridine. Examination immediately after treatment.

Control	144	19	88·3	11·7	100	—
A. 30 mins.	118	31	79·2	20·8	90	10
B. 60 „	121	45	72·9	27·1	82	18
C. 90 „	44	55	44·4	55·6	50	50
D. 120 „	20	77	20·6	79·4	23	77
E. 150 „	10	97	9·4	90·6	10	90
F. 180 „	1	130	0·77	99·23	1	99
G. 210 „	1	106	0·94	99·06	1	99
H. 240 „	0	113	0	100	0	100

Vapour from Ammonia ·880. Examination 1–2 hrs. after treatment.

Controls	44 43	6 7	87	13	100	0
A. 20 secs.	39 40	11 10	79	21	91	9
B. 40 „	26 20	29 35	41·8	58·2	47	53
C. 60 „	2 0 3 1	48 50 47 49	3	97	3	97
D. 80 „	1 1 0 4	49 49 50 46	3	97	3	97
E. 100 „	0 0 0 0	50 50 50 50	0	100	0	100

Table V (*contd.*)

Vapour of pure Pyridine. Examination after 16 hours.

					% calculated on live mites in control	
Time of Exposure	No. Alive	No. Dead	% Alive	% Dead	% Alive	% Dead
Controls	50 40	5 5	90	10	100	0
A. 7·5 mins.	86 89	18 11	85·8	14·2	95	5
B. 15 ,,	132	13	91	9	100	0
C. 22·5 ,,	130	22	85·5	14·5	95	5
D. 30 ,,	81 83	32 17	77	23	85·5	14·5
E. 37·5 ,,	83 85	17 15	84	16	93	7
F. 45 ,,	45	55	45	55	50	50
G. 52·5 ,,	45	55	45	55	50	50
H. 60 ,,	3	97	3	97	3	97
I. 67·5 ,,	7	98	6·6	93·4	7	93
J. 75 ,,	6	94	6	94	7	93
K. 82·5 ,,	6	94	6	94	7	93
L. 90 ,,	0	100	0	100	0	100

Vapour of pure Aniline. Examination immediately after treatment.

Control	86	14	86	14	100	—
A. 60 mins.	81	19	81	19	94	6
B. 120 ,,	43	57	43	57	50	50
C. 180 ,,	5	95	5	95	6	94
D. 240 ,,	2	98	2	98	2	98
E. 300 ,,	1	99	1	99	1	99

which time the vapour had escaped. An inspection of Table V and Fig. 1*a* shows that immediate examination rather understates the effect of the poison. The proportion of live to dead mites was counted in both treated tubes and controls and the percentage of survivors in the tests to the live mites in the controls calculated. This percentage was plotted against time. The results are set out in Table V and Fig. 1*a*.

Fig. 1*a* shows that air saturated with Pyridine is rather more toxic than when saturated with Aniline. This does not mean that weight for weight or molecule for molecule Pyridine is more toxic than Aniline; as the latter having a lower vapour pressure at ordinary temperatures (15°–18° C.) would saturate air with less of that material (weight for weight) in the vapour phase. The curves take the usual sigmoid form characteristic of such reactions. Henderson Smith(4) who studied the toxic action of Phenol on Botrytis spores obtained curves of a similar

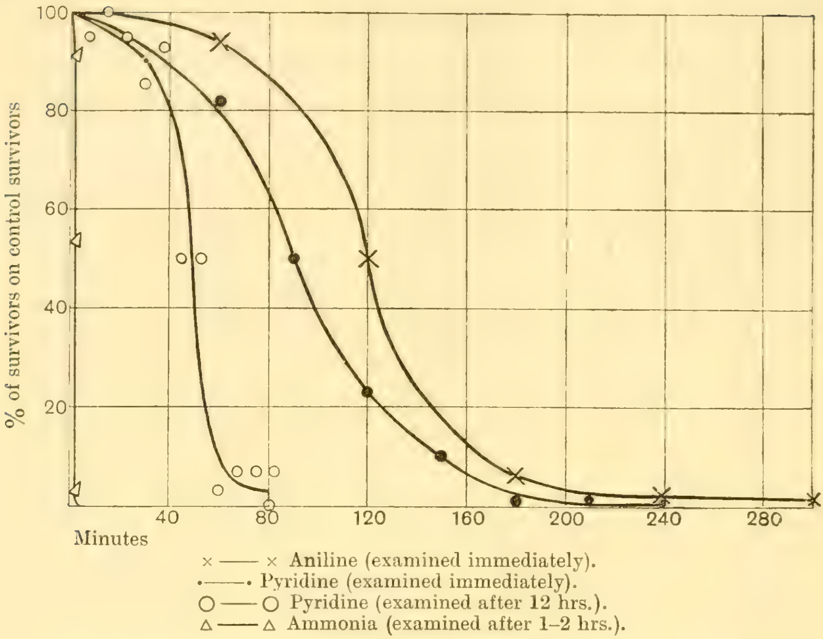


Fig. 1 *a*. Toxicity of vapours of Ammonia, Pyridine and Aniline to mites.

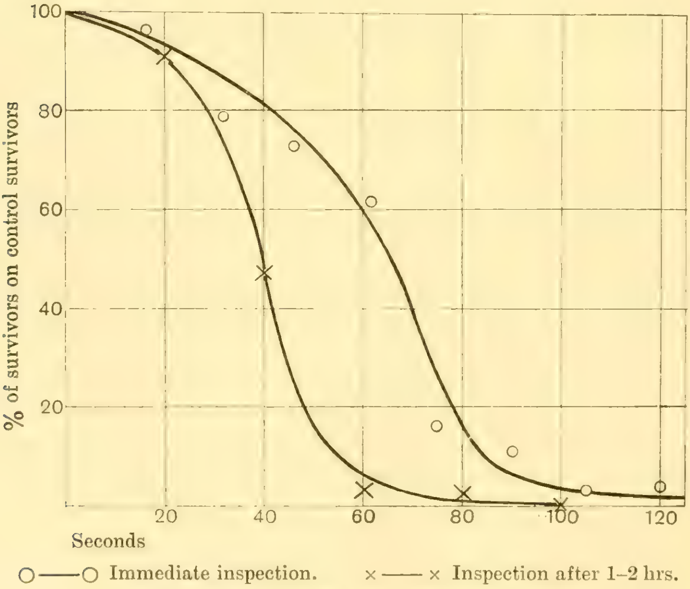


Fig. 1 *b*. Toxicity of Ammonia vapour to mites.

type, and showed that if the strength of the phenol be progressively raised the curve approximates to the logarithmic type, but that both types of curve are explicable by assuming variations of resistance amongst the spores.

In our case, working at the saturation point of poison in air, a similar method was impossible, but even with so rapidly acting a poison as Ammonia the curve obtained was distinctly sigmoid in type when survivors were plotted against time expressed in seconds instead of minutes. Curves of this type would be expected in our case, where we have in an inseparable mixture, adult mites of various ages, larvae and nymphs. The distribution of the resistances varying in all three stages of development would be complex, and the variations so great that with the most highly toxic of materials the survival curve would be of the type obtained. The toxic action of Ammonia proved so rapid that it is not expressible with accuracy on the same scale as that of Pyridine and Aniline, and is put therefore on a second scale in Fig. 1*b*.

In view of the above results it seemed of importance to ascertain whether the considerable toxic action of Pyridine could be regarded as specific. Tattersfield and Roberts(3) showed that molecule for molecule Aniline in the vapour phase was about three times as toxic to wireworms as Pyridine. For this purpose minute but progressively increasing quantities of Pyridine or of Aniline were inserted by means of a graduated capillary pipette into calibrated flasks (1100–1200 c.c.) fitted with lead-lined rubber stoppers and glass hooks, and the cork inserted. When the material had evaporated, mites (about the same number in each case) were introduced in small test tubes closed by silk gauze and attached by wire to the hook and allowed to stand for a period of three hours. This time was convenient as permitting each set of tests to be comfortably finished in one day and as giving nearly 100 per cent. of deaths with a saturated concentration of Pyridine in air. The counts were made in the usual way. The results are shown in Table VI and percentages of survivors plotted against millionths of gramme-molecular concentrations of poison in a litre of air, in Fig. 2.

The curve for Pyridine is distinctly sigmoid in character, indicating that equal increases in concentration do not have a corresponding effect. An increase of dose from 30 to 50 millionths of a gramme-molecule shows little or no increase in toxic action, but an increase of from 50–70 accounts for 75 per cent. of the mites while further increases up to near the saturation point produce effects only very gradually. The curve for Aniline is not complete, as towards the lower end of the curve the flasks are saturated with vapour, the slowing down of the reaction being undoubtedly due to this cause.

Table VI.

Showing Toxic Effect of increasing doses of Pyridine and Aniline to Cheese-mites (T. longior).

Pyridine. Exposure 3 hours. Temp. 16°–18° C.

	Vol. of flask c.c.	c.c. added	c.c. added per 1000 c.c.	Wt. per 1000 c.c. of air	Millionths of gm. mol. per 1000 c.c. of air (approx.)	No. alive	No. dead	% alive	% alive calculated on live mites in control
Control	—	—	—	—	—	117	34	77·5	100
A.	1156	·002	·00173	·0017	21·5	75	25	75	97
B.	1191	·004	·0033	·0032	40	75	25	75	97
C.	1172	·006	·0051	·005	63	32	52	38	49
D.	1192	·008	·0067	·0066	83·5	14	91	13	17
E.	1175	·01	·0085	·0083	105	9	78	10	13
F.	1178	·012	·01	·0098	124	10	101	9	11
G.	1169	·014	·012	·0118	150	2	98	2	3
H.	1183	·016	·0135	·0132	167	3	89	3	4
I.	1158	·018	·0155	·015	190	0	90	0	0

All the mites after treatment with Pyridine extremely sluggish, and appear paralysed.

Aniline. Exposure 3 hours.

Control	—	—	—	—	—	174	62	74	100
A.	1156	·001	·00086	·00086	9	93	51	65	88
B.	1191	·002	·0017	·0017	18	58	80	42	57
C.	1172	·003	·0026	·0026	28	29	85	25·4	34
D.	1192	·004	·0033	·0033	36	32	130	20	27
E.	1175	·005	·0043	·0044	47	27	143	16	21
F.	1178	·006	·005	·005	54	22	137	14	19
L.	1167	·018	·015	·0153	164	25	112	18	25

All the live mites after Aniline treatment more active than in case of Pyridine. All flasks saturated from D downwards before treatment. C doubtful.

On these curves the 50 per cent. survivor points correspond to 21 millionths of a gramme-molecule of Aniline and 63 of Pyridine, indicating that at this point, the most suitable one for purposes of comparison, Aniline is about three times as toxic as Pyridine. Taking the Aniline curve as a whole, the break towards the end due to the fact that from about 35 millionths of a gramme-molecule upwards the air is saturated with vapour, shows that its toxic inefficiency is not due to an intrinsic lack of poisonous properties, but that its low vapour pressure limits to this extent its concentration in air at ordinary temperatures.

From a qualitative point of view Pyridine seems to have an entirely characteristic reaction. In the case of Aniline the survivors up to the 50 per cent. death point are fairly active. With Pyridine, however, from the smallest dose upwards the survivors are obviously seriously incapacitated; they appear to be suffering from motor paralysis and are only just capable of twitching mouth parts and legs. This unfortunately cannot be expressed graphically, but the condition is so marked as to indicate that Pyridine has a very powerful and possibly a specific toxic action on these pests. So small a concentration as ·0017 c.c. in 1000 c.c. of air is capable of almost completely paralysing in three hours nearly 100 per cent. of the mites.

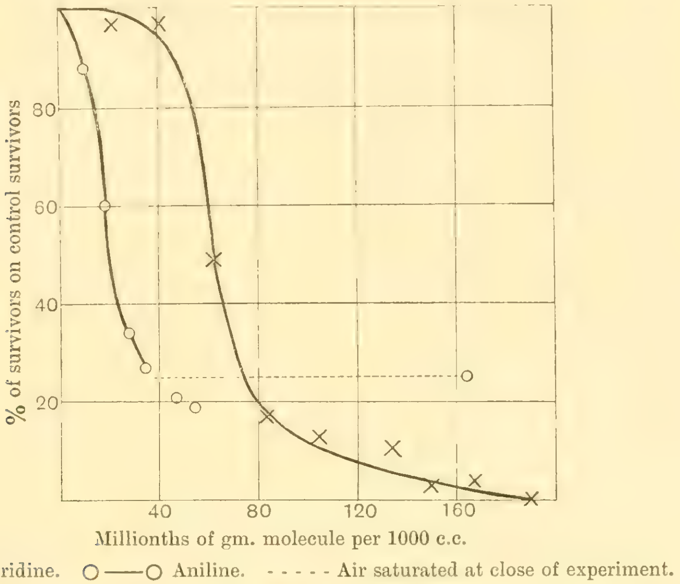


Fig. 2. Toxic effect on mites of increasing concentrations of the vapours of Pyridine and Aniline.

8. TOXIC EFFECT OF PYRIDINE ON FUNGI.

After preliminary trials had shown that Pyridine was successful in eliminating mites a large number of infected cultures were treated with the vapour of Pyridine overnight (16 hours). The infection consisted chiefly of *A. farinae* with a large number of eggs, some *T. longior* while several cultures of *Mucor* were also infected with *G. spinipes* or *G. cadaverum*. The cultures were examined a week after treatment and there was no recovery of mites or hatching out of eggs. They were then subcultured on Czapek's agar and all the subcultures grew and were apparently unaffected by the Pyridine. The six cultures of *Mucor* were, however, contaminated with *Penicillium*. A possible explanation of this seems to be that the mite *Glyciphagus*, present only in these tubes, has long hairs capable of carrying *Penicillium* spores. The cultures treated were of species isolated from the soil and were as follows:

Mucor hiemalis (Wehmer), *Botrytis pyramidalis* (Sacc.), Johnson, *Hormodendrum cladosporioides* (Fres.), Sacc., *Gliocladium penicillioides Corda* (Icon.), *Stachybotrys alternans* Bonord., *Monosporium* sp., *Fusarium*, sp. 1, *Fusarium*, sp. 2, *Penicillium*, sp. 1, *Penicillium*, sp. 2.

Nine other unidentified species including one of the Sphaeropsidales and a Dematiate form were also treated, the total number of cultures being 78. Since this experiment the method of treatment has been used a number of times and has been successful with one possible exception. In the latter case three cultures which had been treated were found some months later to be infected, but as they were among other newly infected cultures it was impossible to tell whether this was due to the failure of the original treatment or to re-infection. It was decided, in view of these results, to carry out a few quantitative experiments on the toxic effect (if any) of Pyridine to some common fungus, in order to ascertain how far this treatment could be carried with safety. The fungus chosen was *Aspergillus niger*, since work on the effect of Pyridine and various organic bases on this organism had been carried out by Brenner (5) and Lutz (6).

Into each of a series of conical flasks of 500 c.c. capacity, 200 c.c. of a suitable liquid medium was introduced, and sterilised. Gradually increasing amounts of pure Pyridine were added, and the flasks inoculated with 5 c.c. spore suspension. After a period the cultures were filtered, thoroughly washed by decantation, dried and weighed. After some preliminary experiments Coons' solution containing double the amounts of all the ingredients was decided upon as giving in a reasonable time a yield of a suitable amount for both washing and weighing purposes[1].

The most rapid and efficient filter was a Gooch crucible used with a pad of cotton-wool and under a not too high vacuum. The Gooch

---

[1] The medium contained in 1000 c.c

	gm.
Magnesium sulphate	0·986
Potass. bi-phosphate	2·720
Asparagin	0·530
Maltose	7·200

crucibles and fungus were then dried for 24 hours at 70° C. and finally at 90°–100° C. to constant weight.

The technique of the method has not yet been completely studied by us, but it proved of sufficient accuracy for the purpose of this investigation. Experiment No. 1 was carried out on an *Aspergillus* sp. isolated from an onion. The cultures were incubated at 26° C. for a period of seven days. Table VII shows that the growth of this fungus is not inhibited until a concentration somewhere between ·318 and ·636 per cent. of Pyridine is reached.

Table VII.

Effect of Pyridine on Aspergillus niger.

Culture solution Coons' double strength 200 c.c. Temp. 26° C.

	Description	Remarks after 3 days	Crop yield after 7 days
1.	·159 % = ·002 gm. mol. Pyridine per 100 c.c.	Growth equal to control	·3914 gm.
2.	·318 % = ·004 „ „ „	Reduced growth; small colonies	·4044 „
3.	·636 % = ·008 „ „ „	No growth	No growth
4.	1·272% = ·016 „ „ „	No growth	No growth
5.	Control	Good growth	·3536 gm.
6.	„	„	·3845 „
7.	„	„	·3942 „
8.	„	„	·3620 „

It was desirable to ascertain whether the toxicity of Pyridine was due to its possessing basic properties either acting directly or indirectly by its effect upon the *p*H value of the medium. Two sets of flasks were used. One set contained gradually increasing doses of pure Pyridine as in the previous experiment, but in this case starting with ·318 per cent. of this compound (= ·004 gm.-mol. per 100 c.c.) and working up by smaller increases to ·636 per cent. The quantities of Pyridine in the second set exactly tallied with those in the first except that before addition the Pyridine solution was brought to a *p*H value of about 4·7 (the same as the medium) by the addition of appropriate amounts of standard sulphuric acid. The culture of *Asp. niger* used was one kindly given to us by the Pure Culture Laboratory at the Lister Institute, No. 594, grown on Czapek's medium and about 7 days old. It proved, unfortunately, rather more susceptible to poison than the one used in the previous test. After inoculation the flasks were set aside in a dark cellar, the temperature of which remained somewhere between 18·5° C. and 19·5° C. Recourse was had to a rather lower temperature as there appeared to be some escape of Pyridine when the cultures were incubated at 25° C. The yields were weighed after a period of three weeks. An inspection of Table VIII brings out with startling clearness the large differences in yield that ensue through the minimising of the toxic effect of Pyridine by the addition of amounts of acid in quantities sufficient partially to neutralise the base.

Table VIII.

Toxicity of Pyridine to Aspergillus niger.

Culture solution Coons' double strength.
Pure Pyridine S.G. ·983 added.
Culture and organism. *Aspergillus niger* from Lister Inst. No. 594.
Period of incubation 21 days.
Temperature of incubation 18°–19° C.

No.	Description of test	Gm. Pyridine added per 100 c.c. medium	Gm.-mol. Pyridine added per 100 c.c.	Yield of fungus gm.
C 1.	Control (no Pyridine)	—	—	·2881
C 2.	Control ,,	—	—	·2655
C 3.	Control ,,	—	—	·2389
P.s. 1.	Pyridine added and acid to adjust pH to 4·7	·31115	·00394	·2102
2.	,, ,, ,, ,, ,,	·3351	·00424	·2164
3.	,, ,, ,, ,, ,,	·3590	·00454	·2051
4.	,, ,, ,, ,, ,,	·3829	·00484	·1885
5.	,, ,, ,, ,, ,,	·4069	·00515	·2293
6.	,, ,, ,, ,, ,,	·4308	·00545	·2242
7.	,, ,, ,, ,, ,,	·4547	·00575	·1922
8.	,, ,, ,, ,, ,,	·4787	·00606	·2119
9.	,, ,, ,, ,, ,,	·5027	·00636	·2205
10.	,, ,, ,, ,, ,,	·5265	·00666	·1218
11.	,, ,, ,, ,, ,,	·5505	·00696	·1666
12.	,, ,, ,, ,, ,,	·5744	·00727	·1792
13.	,, ,, ,, ,, ,,	·5983	·00757	·1320
P. 1.	Pyridine added. No adjustment of pH	·31115	·00394	·0361
2.	,, ,, ,, ,, ,,	·3351	·00424	·0212
3.	,, ,, ,, ,, ,,	·3590	·00454	·0070
4.	,, ,, ,, ,, ,,	·3829	·00484	·0004
5.	,, ,, ,, ,, ,,	·4069	·00515	·0002

As we wished to trace the toxic action of Pyridine completely, and as these results indicated that our initial additions of Pyridine were too large, a fresh series of experiments was set up commencing with an extremely small dose (about ·005 per cent.) and ranging up by small additions to concentrations that experience showed were sufficient to inhibit growth completely. The greatest care was taken to ensure that

Table IX.

Toxicity of Pyridine to Aspergillus niger.

Series III. Culture medium Coons' double strength 200 c.c.
Pure Pyridine (S.G. = ·982) added from capillary pipette.

Culture and organism *Aspergillus niger* from Lister Inst. No. 594. Subculture on Coons' agar.

Age of culture 20 days. Inoculated in 5 c.c. sterile water.

Time of incubation 21 days.

Temperature of incubation 18°–19° C

No.	c.c. of Pyridine per 205 c.c.	Gm. Pyridine per 100 c.c. (originally added)	Gm. Pyridine per 100 c.c. found at end of expt.	Mean gm. Pyridine per 100 c.c.	Gm. yield of fungus	Yield after the addition of standard acid to neutralise Pyridine not re-inoculated
1.	·01	·0048	—	(·0048)	·5074	—
2.	·05	·024	·024	·024	·5251	—
3.	·1	·048	·047	·048	·4817	—
4.	·15	·073	—	(·073)	·4887	—
5.	·2	·095	·096	·095	·4717	—
6.	·25	·119	—	(·119)	·4674	—
7.	·3	·143	·136	·139	·4885	—
8.	·35	·167	—	(·167)	·4644	—
9.	·4	·191	·20	·195	·4676	—
10.	·46	·219	·208	·213	·3899	—
11.	·492	·235	·209	·222	·4540	—
12.	·540	·257	·248	·252	·2699	—
13.	·590	·282	·270	·276	·2103	—
14.	·640	·305	·283	·294	·0862	—
15.	·688	·329	·285	·307	·0218	—
16.	·738	·351	·329	·340	·0152	—
17.	·781	·373	·326	·349	·0091	—
18.	·836	·398	·367	·382	·0034	—
19.	·784	·421	(·3845)[1]	—	Traces	·4857
20.	·934	·445	—	—	,,	·5185
21.	·984	·468	—	—	No growth	·5424
22.	1·034	·491	—	—	,,	·5042
23.	1·082	·515	—	—	,,	·5569
24.	1·132	·539	—	—	,,	·5250
25.	1·18	·561	—	—	,,	·4957
26.	1·23	·585	—	—	,,	·5346
27.	1·28	·608	(·558)[1]	—	,,	·4811
28.	Control 1[2]	—	—	—	·5604	—
29.	,, 2	—	—	—	·5229	Mean
30.	,, 3	—	—	—	·5390	·5404
31.	,, 4	—	—	—	·5231	—
32.	,, 5	—	—	—	·5568	—

[1] Determined after neutralisation of Pyridine by acid and allowing fungus to grow a further three weeks.

[2] Controls gave a precipitate with Iodine in Potass. Iodide equal to ·0025 per cent. Pyridine. This was allowed for.

each lot of medium should be treated in the same way during sterilisation so that no variations in dilution should take place. The flasks were as far as could be judged of the same size and the cotton-wool plugs rolled in the same way and fitting as nearly as possible the necks of the flasks with an equal tightness. Pyridine was added from a capillary pipette, and the flasks inoculated and incubated as in previous series at 18·5°–19·5° C. for a period of 21 days.

At the end of this period they were filtered and after careful washing and drying the yields obtained were weighed. A portion of the filtrate was set aside and the Pyridine estimated by the method of Harvey and Sparks (7) which we had found to give results of fair accuracy. As the medium itself gave a precipitate with Iodine solution in Potassium Iodide, a blank estimation was done on the controls and the amount deducted from that found in the tests. We were thus able to ascertain whether the concentration of Pyridine remained the same throughout the experiment. The means of the amounts of Pyridine added initially and found at the end were taken and plotted against the yields, the curve being drawn freehand through the points.

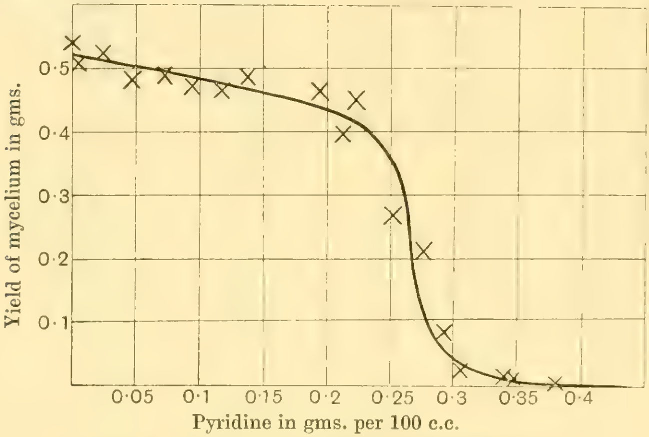


Fig. 3. Toxicity of Pyridine to *Aspergillus niger*.

Table IX and Fig. 3 show that the toxic effect of Pyridine is at first very gradual, the growth yields diminishing very slowly up to a concentration of ·225 per cent. The inhibitive effect of the base from that point onward is, however, increasingly marked and the yields diminish rapidly with small increases in the concentration of Pyridine until at a strength of ·325 per cent. they are almost negligible after which the curve of growth yields tails off very gradually, thus taking a sigmoid shape.

This result corresponds with that obtained by Henderson Smith (4) in his work on the toxic action of phenol on Botrytis spores and it seems probable that a similar explanation should be given in this case, *i.e.* one based upon the variation in resistance of the fungus spores. If it is assumed that this variation is normal and that the spores could be graded according to resistance, then successive grades would contain numbers of spores rising to a maximum in the middle grades and falling again in the last grades. Thus with each successive dose of Pyridine successive grades of spores would fail to germinate. It follows that the middle of the curve is steepest, since with these doses the largest numbers of spores are either killed or their growth inhibited. The toxic effect of the addition of small doses of Pyridine is therefore at first slight then rises to a maximum and falls again as higher concentrations are reached[1].

Table X.

Comparison of Effect of Pyridine and Caustic Soda on growth of Aspergillus *at the same* p*H values.*

No.	Base added	Wt. of base contained in 100 c.c. of medium	*p*H value	Gm. yield of fungus
19.	Pyridine	·429 gm.	6·45	No growth
21.	,,	·476 ,,	6·5	,,
23.	,,	·524 ,,	6·52	,,
25.	,,	·571 ,,	6·55	,,
5.	Caustic soda	·016 ,,	6·45	·4808
6.	,, ,,	·018 ,,	6·5	·4644
7.	,, ,,	·019 ,,	6·52	·4792
8.	,, ,,	·020 ,,	6·55	·4823
C 1.	Control	—	4·75	·4553
C 2.	,,	—	4·75	·4345

[1] As the medium used was slightly acid (*p*H 4·45–4·75) it might be considered that at the lower concentrations of Pyridine its toxicity might be seriously lessened by partial neutralisation. Mr E. M. Crowther kindly determined for us the effect upon the *p*H value of our medium of progressively increasing additions of Pyridine and so the amounts of undissociated base present. With an addition of ·01 gm. of Pyridine to 100 c.c. of medium 53 per cent. was present as free base, while additions from ·1 to ·9 gm. to 100 c.c. of medium gave amounts of free undissociated Pyridine ranging from 82–92 per cent. of the amounts added. The effect upon the curve is to displace it slightly to the left, but not fundamentally to alter its character. We are aware of the fact that *Aspergillus niger* may during growth give rise to notable amounts of acid and that in tracing out an accurate graph of the toxicity of Pyridine to this fungus the amounts of free base before and after the experiment should be determined. This, however, was outside the scope of these preliminary experiments, which were intended to ascertain to what extent a common fungus could tolerate this base when added to a synthetic medium.

As previously stated it was considered that these results might be due to the alteration in pH values of the medium on the addition of Pyridine. The medium used has a pH value of about 4·75, which the addition of ·429 per cent. of Pyridine brought up to 6·45. To test this point a series of experiments was set up in which the pH's of the medium were adjusted by means of $N/10$ Sodium Hydrate to those obtained in the flasks in which the higher concentrations of Pyridine inhibited growth. It will be seen from Table X that the effect of increasing the pH from 4·75 to 6·55 is very small. The alteration of pH plays, therefore, an insignificant part. One other interesting point arising from these experiments is that the effect of Pyridine is to inhibit the germination of the spores rather than to kill, at any rate all of them, outright. After the yields in Series III, Table IX, had been weighed, additions of standard sulphuric acid were made to the flasks 21–27 where no growth was visible: within two days the spores in these flasks had begun to germinate and growth took place at a rapid rate. After standing for three weeks the yields were weighed, the results being set forth in the last column of Table IX. They are of the same order as those given by the controls during the previous three weeks. At the end of this period the Pyridine remaining in two of the experiments (19 and 27) was determined. The amounts found are expressed in brackets in column 4.

Lutz (6) has stated that in the presence of some other form of assimilable nitrogen, Pyridine may act as a food to fungi. Although our experiments were not set up to investigate this point and must not be regarded as final, for this particular fungus (*Aspergillus niger*) we have not obtained any evidence of Pyridine acting as a stimulant to fungal growth. However small an addition of this base might be made there has never been shown an increase in the yield which could be considered outside the margin of error of the experiment. There is undoubtedly towards the end of the series in Table IX a loss of Pyridine, which however cannot be accounted for by assimilation, being probably due to volatilisation as it is greater as the amount of growth diminishes. Moreover, the deficiency in the amount of Pyridine found after its neutralisation and allowing the *Aspergillus* to grow for a further three weeks is of the same order as that found in the flasks where at the end of three weeks and before neutralisation the growth had been small.

The amount of Pyridine absorbed by culture media from an atmosphere saturated with its vapour is about 4 per cent. in sixteen hours. This is much more than a toxic dose. Subculturing after treatment, especially of fungi growing in liquid media, is therefore essential.

9. DISCUSSION OF RESULTS.

Toxicity of Pyridine and Ammonia to Mites.

Pyridine is shown to have a considerable toxicity to mites and while its effect upon fungi (in the small doses necessary to kill mites) is practically nil, in continually increasing doses it becomes more marked until a concentration is reached at which germination and growth are completely inhibited. The toxicity to mites is surprising as it has generally been assumed that the toxicological action of Pyridine to all living organisms is not marked.

Pyridine and the various monacid ammonium bases have been the subject of considerable toxicological research, either because of their occurrence as groups in the molecular structure of many well-known and widely used alkaloids (*e.g.* Nicotine) or because of their close similarity to them in physiological action.

Brunton and Tunnicliffe (8) have shown that on frogs, Pyridine has, in relatively small doses, a general narcotic action, that its paralysing action on motor nerve endings is of the slightest and that its action is almost wholly confined to the sensory part of the nervous system. They came to the conclusion that Pyridine, compared with its derivatives, is not an active poison, a conclusion that would hardly be expected when the very marked stability of Pyridine is borne in mind. From its close relationship chemically to Nicotine, one would expect a fairly high insecticidal value, yet Pyridine has proved itself of little use in this respect.

Fryer (9) states that after a large number of tests the results have proved in all cases disappointing. The Entomologist to the United States Dept. of Agriculture (10) confirms this and reports that while the compounds most highly poisonous to insects are to be found among the organic nitrogen derivatives the toxic value of Pyridine is small. Tattersfield and Roberts (3) found that to wireworms, Pyridine was less potent as an insecticide than any other of the organic bases tested.

Although the present results do not definitely prove that Pyridine is a compound of *high* specific toxicity to mites, they do indicate that it possesses a toxic action which is much greater than experience would lead us to expect. We were only able to compare it critically with Aniline, a comparison which led to the conclusion that the low vapour pressure of the latter compound tended to put a limit on its toxicity, but that molecule for molecule in the same time it was more poisonous from a quantitative point of view than Pyridine. On the other hand in very minute doses Pyridine had a most profound narcotic effect, inhibiting all the larger movements and leading to almost complete paralysis.

The great toxic action of Ammonia is not surprising, for it is natural to expect such a strongly irritant substance to be highly poisonous to lower forms of animal life.

Toxicity of Ammonia and Pyridine to Fungi.

The toxicity of Pyridine to lower forms of plant life has been the subject of some investigation. The views expressed although somewhat discrepant generally lean towards the opinion of its comparatively low toxic properties. Morgan and Cooper(11) state that of many monacid organic bases they tested the bactericidal properties of Pyridine were the least. Lutz (6) has stated that it may act under certain conditions as a food, a conclusion not borne out by the experiments described above, but it must be recognised that very special conditions as to media and organism may be required for the feeding effect of Pyridine to manifest itself.

Our experiments do not definitely indicate the position of Pyridine in the toxic scale as far as fungi are concerned, but we lean to the view that it is not high. This is not easy to understand, for the compound is inert and its basic properties weak. The latter fact, if the views of Newton Harvey (12) are correct, should indicate a rather high toxicity. This investigator points out that weak bases penetrate cell walls with greater rapidity than strong bases such as Caustic Soda, and that penetration is of the first importance in determining toxicity. On the other hand, another important and countervailing factor is dissociation, the least dissociated bases being least toxic.

As Pyridine is a weak base and very slightly dissociated its toxic properties might be low despite its penetrating power. It is outside the purview of the present investigation to explore this problem, but the rate of penetration of cell walls by chemical compounds is one of fundamental importance in the consideration of fungicidal and insecticidal problems and further investigation along these lines is contemplated. Our results show that fairly high doses such as ·5–·6 per cent. of Pyridine may inhibit germination and growth, and it is probable, although no proof is here advanced, that this is due to the Pyridine readily permeating the cell. These spores, however, will grow if the base is neutralised by acid, the Pyridine in all probability diffusing out of the cell with readiness as soon as the diffusion gradient is modified in a reverse way by the addition of the acid. Our experiments show that what little toxic properties Pyridine may have, it possesses chiefly in virtue of its basic nature. Its salts are hardly poisonous at all either because the acid ion prevents the migration and penetration of the cell wall by the pyridineum ion, or if the salt of Pyridine does penetrate its toxic properties within the cell are very slight. The toxicity of Pyridine does not arise out of its modification of the *p*H value of the medium but would seem in some way to depend upon a special relationship of the cell to the Pyridine molecule as a base.

10. PRACTICAL APPLICATION OF METHOD.

The following method has been used in the treatment of mite infested fungus cultures with Pyridine. A large bell-jar of about 20 litres capacity is inverted and in the bottom is placed a flat dish containing about 20 c.c. of commercial Pyridine and covered by a wire gauze. The infested cultures, without removing the cotton-wool plugs, are placed in the bell-jar for 16 hours (overnight) and the jar is closed with a glass plate which should be luted down with clay or plasticine. Subcultures taken from the tubes after the above treatment have proved free from the infesting mites, except in one example described above, where some eggs appear to have survived the above treatment, so that in the case of very bad infestations or in very cold weather it may be advisable either to expose the tubes for 48 hours or to give two exposures of 16 hours duration separated by a period of fourteen to sixteen days. The latter method allows any unkilled eggs to hatch, the very susceptible larvae being rapidly poisoned by the second exposure to the vapour of Pyridine. Owing to the rather disagreeable odour of Pyridine it is advisable to carry out the treatment either in a good fume cupboard or outside the laboratory.

Strong Ammonia can be used for cleaning out laboratory apparatus. Its toxic properties to mites are exceedingly great, but as it has a slight but definitely deleterious effect upon some fungi, it is advisable to limit its use to apparatus when its vapour will not play for any prolonged period upon mycological cultures.

Our best thanks are due to Mr H. M. Morris, M.Sc., for much valuable advice and for identifying the species of mites, and to Mr E. M. Crowther, M.Sc., for the determination of the *p*H values of our media.

11. SUMMARY AND CONCLUSIONS.

1. Mites are a serious pest of fungus cultures. The species that most frequently occur are *Aleurobius farinae* and *Tyroglyphus longior* with an occasional infestation with *Glyciphagus cadaverum*.

2. They can be controlled by exposing the cultures to the vapour of Pyridine, after which treatment the fungi can be subcultured safely. An exact description of the application of the method is given on p. 239. (Commercial Pyridine is as effective as the pure material.)

3. If these pests occur in laboratory apparatus they can be eliminated by the application of strong Ammonia. Ammonia and its vapour are very rapidly effective against mites, but they should not be allowed to come into contact with cultures of fungi for too long a period of time in too high a concentration.

4. Pyridine is shown to have a slight toxic action to fungi, and to inhibit growth completely in certain concentrations which, however, are not at all likely to be objectionable in practice, especially if the treated cultures are subcultured.

5. A brief analysis of the toxic action of Pyridine on both Mites and Fungi is given.

(*a*) In the case of Mites minute doses have so powerful a paralysing action as to render it probable that Pyridine is specific in its toxic effect to these pests.

(*b*) In the case of Fungi, the action of Pyridine upon the germination and growth of *Aspergillus niger* was closely studied. It is shown that up to about ·25 per cent., Pyridine has apparently very little toxic action and no feeding effect, but that above this concentration the toxicity increases with great rapidity. It is shown, however, that the toxic action is one of inhibition of germination and that the neutralisation of the base up to 0·6 per cent., the highest concentration tested by us (even though spores have been exposed to its action for three weeks), permits growth to take place rapidly. Pyridine acts chiefly as a poison through its basic properties but not by the change in the *p*H of the medium which ensues on its addition.

REFERENCES.

(1) Eales, N. B. (1917). *Ann. App. Biol.* iv. 29.
(2) Newstead, R. and Duvall, H. M. (1919). *Rev. App. Ent.* **A7**, 91.
—— (1918). Roy. Soc. Rept. of Grain Pests (War) Comm. No. 2.
(3) Tattersfield, F. and Roberts, A. W. R. (1920). *J. of Agr. Sci.* x. 199.
(4) Henderson Smith, J. (1921). *Ann. App. Biol.* viii. 27.
(5) Brenner, W. (1914). *Centr. Bakt.* Abt. 2, Bd. 40, 555.
(6) Lutz, L. (1905). *Bull. de la Soc. Bot. de France*, lii. 194.
(7) Harvey, T. F. and Sparks, C. F. (1918). *J. Soc. Chem. Ind.* xxxvii. 41T.
(8) Brunton, T. L. and Tunnicliffe, F. W. *J. Physiol.* xvii. 272.
(9) Fryer, P. J. (1920). *Insect Pest and Fungus Diseases* (p. 445).
(10) Rept. of the Entomologist U.S. Dept. of Agriculture, Washington, Aug. 1, 1921.
(11) Morgan, G. I. and Cooper, E. A. (1912). 8th Inst. Congr. of App. Chem. **xix.** 243.
(12) Newton Harvey, E. Publ. Carnegie Inst. of Washington, No. 183, p. 131.

(*Received July* 19*th*, 1922.)

ON THE DEVELOPMENT OF A STANDARDISED AGAR MEDIUM FOR COUNTING SOIL BACTERIA, WITH ESPECIAL REGARD TO THE REPRESSION OF SPREADING COLONIES[1]

By H. G. THORNTON.

(*From the Bacteriological Department, Rothamsted Experimental Station.*)

(With 13 Text-figures.)

1. Introduction.

The recent developments in our knowledge of soil organisms emphasise the necessity for quantitative methods of research on this subject.

An accurate method of estimating the rise and fall of bacterial numbers in the soil, must underlie the study of soil micro-organisms, in their relations both to each other and to the fertility of the soil. The present work was undertaken with the object of increasing the accuracy of the plate method for counting soil bacteria.

When considering this method, it is necessary to bear in mind what kind of information should be obtainable by its use. The method has strict limitations. Thus, not all the physiological groups of soil bacteria are able to develop and produce colonies on any single medium. Yet the medium used should enable the great majority of bacteria in the soil to form colonies upon it, and in speaking of bacterial numbers we usually recognise the exclusion of those few groups which need special media. There are, however, other sources of error in the method, which still further reduce the number of colonies which appear on the platings. Chief amongst these would seem to be the adherence of bacteria to soil particles, the death of some of the organisms during the diluting and plating processes, and the interference between colonies on the plate. The under-estimation of bacterial numbers obtained by the plate method is well shown by work, such as that of Breed and Stocking(1) on the

---

[1] A grant in aid of publication has been received for this communication.

bacterial numbers in milk, in which the numbers obtained by the plate method were compared with those derived from direct counts made under the microscope. The plate method, therefore, cannot tell us the total numbers of bacteria in a soil sample, but it affords a means of comparing the numbers in two or more samples, by enabling us to count a percentage of the total numbers. To make comparison possible, this percentage must not appreciably vary.

The sources of error in the method, which are not preventable, must therefore be standardised, so that they will affect the calculated numbers to a constant degree. Thus, when comparing the bacterial numbers in different soil types by this method, errors may be introduced by the adherence of groups of bacteria to soil particles. The extent to which this occurs is not at present known, but it is likely to vary in different types of soil. In working with a single soil type, however, this source of error is likely to remain constant and therefore loses its primary importance. The close agreement that we have found (2) between bacterial numbers calculated by this method from parallel soil samples taken at Rothamsted seems to indicate that this is the case. The percentages of organisms which are lost in the diluting and plating process, can be rendered sufficiently constant by careful standardisation of the technique. A variation in numbers obtained, resulting from random sampling, is necessarily involved in making the dilutions. This variation can, however, be calculated and due allowance made for it.

The remaining sources of error are connected with the medium used in the plating and with the development of the colonies therein. The medium appeared to be so great a cause of variation as to render its investigation a matter of first importance, and a necessary prelude to the study of the other factors before mentioned. Its investigation has therefore formed the subject of the present work.

The qualities to be looked for in an ideal count medium have been described by Conn (3). For the purpose of the general bacterial count, constancy in the results obtained with a medium is by far its most important property. The medium should be exactly reproducible by different workers or by the same worker at different times. Also, a suspension of soil, if plated on different samples of the medium, should give rise to the numbers of colonies differing only within the limits of random sampling variance.

Constancy in the results obtained with a medium depends mainly on the following features.

A. The composition of the medium must be constant.

B. There must be as little interference between the developing colonies as possible. For example, the rapid growth of spreading colonies must be checked.

C. The medium must not encourage rapid growth of fungi.

D. Its reaction must vary but slightly.

If the composition of a medium is to be sufficiently constant, it must not contain food constituents whose composition varies. It is in this respect that most of the older media failed, for the earlier work was carried out upon media containing peptone, meat extract, "Nahrstoff Heyden" or some such food supply of uncertain composition.

The first important development from this stage consisted in simplifying the medium and greatly reducing the content of organic matter. Thus Fischer(4) tried a medium containing only soil extract and phosphate as food substances, and Temple(5) used 0·1 per cent. peptone as the sole source of organic matter. It was found that this reduction in organic matter lessened the growth of spreading colonies to some extent and allowed higher counts to be obtained.

At about this time there arose the idea of the "synthetic medium" in which only pure chemical compounds were used as food constituents. Fischer(4) describes such a medium, and Lipman and Brown(6) tried agar media containing dextrose as the source of energy material and KNO_3 of $(NH_4)_3SO_4$ as the nitrogen supply. The medium which they finally developed, however, contained peptone and thus was not truly a "synthetic medium." Brown also tried media with casein, urea, albumen, and asparagine as sources of nitrogen. With the same idea, Conn (3), in 1914, developed an agar medium to which nitrogen was added as ammonium phosphate and sodium asparaginate.

Although past work has thus shown that food substances can be provided in the form of definite chemical compounds, there is great difficulty in obtaining a gel-producing constituent of constant composition. Silicic acid is unsuitable for general use for this purpose. The present author carried out some experiments with cerium hydrate gel, at the suggestion of Dr Emil Hatschek, but was not successful in using it for plating. It would, therefore, appear that an organic colloid such as agar or gelatine is alone suitable for this purpose. In the constancy of the results obtained with it, agar is far superior to gelatine, both on account of its less variable composition and because of its comparatively low feeding value to bacteria. This is illustrated in the following experiment (see Table I). Media were made up in which the food constituents of Conn's sodium asparaginate medium(3), were added to four different brands of agar and three different brands of gelatine. The agar media were sterilised in the autoclave at 15 lbs. for 15 minutes and the gelatine for 20 minutes at 100° C. on three consecutive days. The media A and B were made up with the same agar, all the constituents being separately weighed out in each case, in order to test the error involved in preparing the media. This error appears to be negligible. The media were tested with regard to their acidity and their capacity for colony development.

The H-ion concentration was measured by the indicator method of Clark and Lubs, both before and after sterilisation.

The greater constancy of agar is noticeable both in the original reaction and in the smaller change which occurs on sterilisation. To test the colony development, six parallel platings were poured with each medium, from a single diluted suspension of Rothamsted soil. The results again show the advantage of agar in that it is less variable in its effects, a feature evidently connected with the greater constancy in reaction between samples.

Table I.

Variability between Samples of Agar and Gelatine.

Medium	Sample of agar or gelatine	*p*H value before sterilising	*p*H value after sterilising	Bacterial colonies on each plate	Mean no. of colonies
A.	Sample 1. Shred agar	6·7	6·6	33, 32, 31, 30, 30, 28	30·7
B.	Same. Salts weighed separately	6·7	6·6	34, 33, 32, 30, 30, 29	31·3
C.	Sample 2. Shred agar	6·8	6·65	47, 35, 35, 34, 33, 27	35·2
D.	,, 3. Powdered agar	6·8	6·6	35, 34, 34, 33, 28, 27	31·8
E	,, 4. Powdered agar	6·8	6·5	50, 46, 45, 42, 40, 38	43·5
F.	,, 5. Gelatine	6·0	5·7	16, 14, 14, 12, 10, 6	12·0
G.	,, 6. Gelatine	6·4	5·9	15, 13, 13, 12, 11, 8	12·0
H.	,, 7. Gelatine	5·4	5·2	8, 7, 6, 5, 5, 4,	5·8

Sample E in the above experiment was an obviously impure agar powder such as would not have been used in routine work. The somewhat abnormal results obtained with it, however, show the advisability of employing some method of washing the agar before use. Fellers(7) found that agar contained compounds of Ca, Mg, S and N which were soluble in 0·5 per cent. HCl. He also observed that agar could support a slight growth of bacteria which produced ammonia therefrom. Several methods of washing and purifying agar have been tried by various authors. Thus Fellers(7) made a sol of 5 per cent. agar in distilled water and precipitated this in alcohol. Cunningham(8) washed agar in dilute acid, filtered it through cotton-wool in an autoclave, and dried the filtered product in an oven. It is claimed that this product is purified and that filtration of the media prepared from it is greatly facilitated. Most workers, however, have washed the agar either with water or dilute acid and have then dried it, while in the shred condition. To test the advantages of washed agar in counting technique, some agar was washed and filtered by Cunningham's method, and a second quantity was washed while in the shred condition in 0·1 per cent. H_2SO_4 for 10 minutes at 15° C., rinsed free from acid by continued changes of water, and dried. A medium, having the following composition, was made up with the two samples of agar and with unwashed agar as a control.

Distilled water	1000 c.c.	CaCl	0·1 gm.
Agar	15 gm.	KCl	0·1 ,,
K_2HPO_4	1 ,,	Dextrose	0·5 ,,
$MgSO_4.7H_2O$	0·2 ,,	Asparagine	0·5 ,

Each of the three media was tested (*A*) without filtering, (*B*) after filtering at 100° C., (*C*) with the salts filtered before adding the agar, which was not filtered. The acidity was adjusted before autoclaving. In each medium the change of reaction during sterilisation in the autoclave was tested, and platings of a single diluted suspension of Rothamsted soil were made to test its capacity for allowing colony development. The data obtained are shown in Table II.

Table II.

Effect of Acid washed Agar.

Treatment	*p*H value before adjustment	*p*H value adjusted to	*p*H value after autoclaving	Bacterial colonies on each plate	Mean no. of colonies
Agar unwashed, medium not filtered	6·7	7·3	7·0	90, 87, 85, 82, 78, 73	82·5
Agar unwashed, medium filtered	6·7	7·3	7·0	74, 74, 72, 69, 65, 63	69·5
Agar unwashed, salts filtered	6·7	7·3	7·0	95, 80, 72, 70, 68, 68	75·5
Cunningham treatment, medium not filtered ...	6·45	7·2	6·7	53, 52, 52, 48, 47, Sp.*	50·4
Cunningham treatment, medium filtered ...	6·45	7·2	6·7	69, 63, 54, Sp. Sp. Sp.	62·0
Cunningham treatment, salts filtered	6·4	7·2	6·7	62, 61, 55, 53, Sp. Sp.	57·8
Acid washed and dried, medium not filtered ...	6·6	7·2	6·8	82, 75, 70, 68, 65, 58	69·6
Acid washed and dried, medium filtered ...	7·2	7·2	6·9	83, 77, 73, 71, 69, 58	71·8
Acid washed and dried, salts filtered	6·5	7·2	6·8	80, 76, 75, 64, 60, 59	69·0

* Sp.: platings lost through spreading organisms.

It will be seen that Cunningham's method of washing and filtering has produced undesirable changes in the agar. The alteration in reaction during autoclaving has been increased, while the number of colonies which develop is distinctly lowered and spreading colonies are encouraged.

Taking the media made up with agar washed in the shred condition it will be seen that in the filtered medium, the colony development is unaffected by the washing in acid but that when the medium is not filtered the unwashed agar permits a rather higher number of colonies to grow. In making up the medium for routine work, however, filtering is necessary, so that the washing will not produce a harmful effect on the development of colonies. As a result of many trials it has been found that agar washed in acid, while in the shred condition, gives more regular results than unwashed agar.

The process of washing in acid has the further advantage, also observed by Cunningham (8), when using his method, that it renders filtration of the medium easier and more rapid. For example, the medium used in the last experiment was made up with unwashed agar and with shred agar washed in 0·1 per cent. acid. The time taken to filter 200 c.c. of medium through filter paper in a warm filter funnel was recorded. It was found that washed agar medium passed through the filter paper in 55 minutes while the unwashed agar medium took 2 hours to pass through.

Experiments with various strengths of acid for use in the washing have resulted in the adoption of a routine technique in which the agar shred is soaked in 0·05 per cent. H_2SO_4[1] for 15 minutes at room temperature, washed in water till acid free, and then dried.

Although the difference in composition between samples of agar may be lessened by washing in acid, yet the removal of impurities is not complete. The effect of these varying impurities must therefore be neutralised. Small quantities of organic impurities, such as occur in the washed agar, are unlikely to influence bacterial growth in a medium already richly supplied with organic and nitrogenous food substances. In a similar manner, the influence of traces of Ca, Mg, S, etc., may be masked by the addition to the medium of quantities of these substances in excess of the bacterial requirement. The necessity of this addition in a medium from which standard results are expected, is sometimes overlooked. This is the case, for example, in some of the "simple" media that have been used both for bacteria and fungi, where reliance is placed on such variable impurities as may be present in the agar, to supply the electrolytes needed for growth. In the present work the Ca, Mg and S salts used in Conn's sodium asparaginate agar (3) have been employed with the addition of NaCl as a source of sodium.

---

[1] For some time 0·5 per cent. acid was used, but it was sometimes found that this affected the gel formation of the agar.

2. THE SPREADING GROWTH OF ORGANISMS ON AGAR PLATES.

Unfortunately agar, when used in a count medium, has one defect that is so serious as to have deterred some workers from its use. Certain commonly occurring soil bacteria form rapidly spreading surface colonies on agar, which, in many platings, cover the agar surface and inhibit or interfere with the development of other colonies. These organisms are so abundant in Rothamsted soil that on meat-extract peptone agar a large percentage of platings are spoilt, and accurate bacterial counts are impossible on such a medium. If the amount of organic nitrogen compounds in the medium be reduced, there is less growth of the spreading organisms. The fact has long been realised and led to the development of such media as Lipman and Brown's agar medium (6), containing only 0·05 per cent. peptone. However, considerable "spreading" still takes place on such media as the above. Conn (3) noticed this fact, which I have also observed with this and with other peptone media. Less "spreading" seemed to occur on media containing simpler organic nitrogen compounds, such as Conn's sodium asparaginate agar. This indicates that a mere reduction in the amount of organic nitrogen in the medium is not an efficient means of checking spreading colonies, but that the nature of the compounds used is of importance. A more exact knowledge of the conditions which control the growth of spreading colonies, and especially of the effect on them of the composition of the medium, appeared necessary.

It was therefore decided to study the behaviour in pure culture of an organism which formed spreading colonies, in the hope that the knowledge thus obtained would enable a medium to be developed upon which the formation of spreading colonies would be restricted. By far the most abundant of these spreading organisms in Rothamsted soil is spore-forming bacillus which appears to be similar to *B. dendroides* described by Holzmuller (9) in his paper "Die Gruppe des Bacillus mycoides." The organism, however, would appear rather to belong to the *B. subtilis* group. The strain here used has the following characters[1].

---

[1] I am indebted to Mr P. H. H. Gray for having worked out the characters of the organism in this laboratory.

MORPHOLOGY. (A) *Vegetative Cells.* (Nutrient agar, Conn, 2 days incubation at 20° C.) The organism consists of short rods, lying singly or in pairs and short chains. Some long rods, up to 10μ occur. Size of majority $4 \times 0{\cdot}5\mu$. The rods are actively motile, and bear 6 to 15 long undulating flagella, which are peritrichous. The organism is gram positive, and takes readily all the usual stains.

(B) *Spore formation.* (Nutrient agar, 4 days at 20° C.) Sporangia consist of slightly thickened rods often in chains. Endospores central in position, elliptical, size of majority $1{\cdot}25 \times 0{\cdot}75\mu$.

CULTURAL CHARACTERS. (A) *Agar stroke.* (2 days at 30° C.) At first filiform, later (4 days), flat and spreading. Smooth glistening surface. Growth opaque and whitish.

(B) *Gelatine stab.* (2 days at 20° C.) Growth best at surface. Line of puncture filiform. Liquefaction commences in 2 days and becomes napiform. After 30 days, depth of liquefaction is about 25 mm.

(C) *Potato.* (2 days at 30° C.) Abundant, viscid growth of whitish colour. Surface dull and wrinkled. Potato discoloured brown.

(D) *Nutrient Broth.* (2 days at 35° C.) Surface pellicle formed. Liquid slightly clouded. No sediment. No odour.

(E) *Agar Colonies.* (2 days at 30° C.) Very rapid growth. Colony formation described below.

(F) *Gelatine Colonies.* (2 days at 20° C.) Circular with entire edges. Saucer-shaped liquefaction. Rather slow growth.

PHYSIOLOGY. (A) *Fermentation.* (Fermentation tubes containing nutrient broth + 1 per cent. of the compound indicated. Incubation 4 days at 30° C.)

Dextrose. Reaction acid (no gas).
Saccharose. Reaction acid (no gas).
Lactose. Reaction alkaline (no gas).
Glycerine. Reaction alkaline (no gas).

(B) *Diastatic Action.* (Starch agar plates incubated at 30° C.) Action strong. Breadth of clear zone in 5 days, 5 to 10 mm.

(C) *Litmus Milk.* (10 days at 30° C.) No coagulation. No change in reaction or litmus reduction.

(D) *Indol Formation.* Negative.

(E) *Nitrate Reduction.* (Nutrient broth + 0·1 per cent. KNO_3 incubated at 30° C.) Nitrite present in two days—no gas in 10 days.

(F) *Chromogenesis.* Negative.

(G) The organism is aerobic. Its optimum temperature for growth is about 35° C.

Before investigating the action of varied external conditions on the "spreading," the process by which the organism produces the normal spreading colony on the surface of meat-extract peptone agar was carefully studied. The germination of spores and the early stages in the division and grouping of the cells were observed by means of the agar block technique described by Hill (10).

In order to observe the formation and subsequent spreading of a colony, platings of sterile nutrient agar were poured and were inoculated at the surface with spores of *B. dendroides*. The plates were incubated at 37° C., and were examined at short intervals under a $\frac{1}{6}$-inch objective. In this way the development of a surface colony could be observed on the actual plating. Owing to the extreme rapidity with which *B. dendroides* grows, no difficulty was experienced from air contaminations, which had no time to develop during the short period concerned.

When incubated at 37° C. on the surface of nutrient agar, the spores germinate in about 45 minutes, producing rods which are at first non-motile. The rods divide rapidly and the daughter cells do not form chains but come to lie side by side so as to form packets of four to six cells. A young colony at this stage has mosaic-like appearance under a low power, owing to the packets of rods lying at divergent angles. The formation of these packets is not uncommon in other organisms and is described and illustrated by a number of authors. Their production is probably conditioned by surface tension and has an important influence on the development of the colony.

At about this time a water film, covering the colony, becomes noticeable. It seems probable that this is derived from the saturated atmosphere covering the agar film, the young colony acting as a point upon which condensation occurs. The surface growth of the organism possesses the power of retarding the absorption of this water by the underlying agar. The following experiment illustrates this. Droplets of distilled water were placed on a portion of the agar over which a colony was spreading and other drops of similar volume upon a sterile portion of the plating. The former took four to six times as long to disappear as the latter. The time taken for water to be absorbed was found to vary according to the thickness of the agar film and other conditions but was always several times greater within the area of a branching colony than outside it. The absorption of water within the colony area was found to be retarded not only where the surface was entirely covered with bacterial growth but also in places where a large fraction of the surface consisted of apparently uncovered agar lying between branches of the colony. It seems, therefore, that the retention of the water cannot be due merely to the closely packed bacteria separating it from the underlying agar.

Two explanations of these facts suggest themselves. Either substances that hinder the access of the water to the agar may cover the surface in neighbourhood of the bacterial growth, or else the organisms may produce a local change in the agar gel such that its capacity to absorb water is reduced. The latter hypothesis can be tested by growing the organism on the lower surface of a film of agar and measuring the rate of absorption of water drops by the agar immediately above the growth and elsewhere. Under these conditions, any substances produced by the organism on the surface of growth will no longer lie between the agar and the water drops, whereas a change in the absorption capacity of the agar would reveal itself, and, if found to occur, the depth to which the gel is affected could be observed by varying the thickness of the agar film. This test was applied by the following experiment. A very small droplet of 12 per cent. gelatine was placed in the centre of each of six sterile petri dishes, and each droplet was inoculated with spores of *B. dendroides* and allowed to set. Nutrient agar medium, melted and cooled to 42° C., was then poured into each dish. The gelatine kept the spores adhering to the glass so that all subsequent growth of the organism took place beneath the agar film. By varying the quantity of agar in the dish, the thickness of the film was varied. Quantities of from 5 to 20 c.c. were used, giving films of from about 1 to 3 mm. in thickness. After about 48 hours incubation there was good growth along the bottom of each plating. Drops of distilled water, 0·02 c.c. in volume, were placed on the agar surface in each dish, both above the bacterial growth, and outside the colony area, and their rates of absorption by the agar were measured. On all the platings, the water was absorbed at an equal rate above the bacterial growth and outside this area. It appears, therefore, that there is no alteration in the water absorbing capacity of the agar gel in the neighbourhood of the colony. We must therefore conclude that the retention of moisture on the surface about the bacterial growth is due to some secretion, probably of a mucilaginous nature which hinders access of the water to the underlying agar.

The rods, that are at first produced by germination of the spores, are non-motile, but soon peritrichous flagella are developed. These have mean length of about 10μ, and are undulating. In the young colony the cells bear 8 to 15 flagella, but this number appears to be reduced to about half in the older growths.

As the moisture appears, a slow motility can be observed in the colony, the rods sliding over one another and slowly pushing outward the edge of the colony. Single rods do not appear capable of overcoming the surface tension at the edge of the water film. They have many times been observed pushing outward but do not force their way out from the colony[1]. Packets of six or more cells, however, are able slowly to press outward the edge of the water film. Consequently, where a packet of cells lies at the edge of the colony, in such a manner that the rods lie radially, or at right angles to the film edge, they may often be observed to force their way outward producing a small promontory (Fig. 1). But where the outer packets lie so that the cells are oriented tangentially, they do not press away from the centre. The out-pushing of the colony edge is therefore discontinuous so that the colony becomes irregular or lobate. As the water film becomes thicker, the cells move about more actively, and, in the interior of the colony, they often lose their arrangement in packets, and where there is most moisture a streaming movement of cells may be observed. In each projection of the colony edge, the cells tend to swim outward and to collect at the distal extremity,

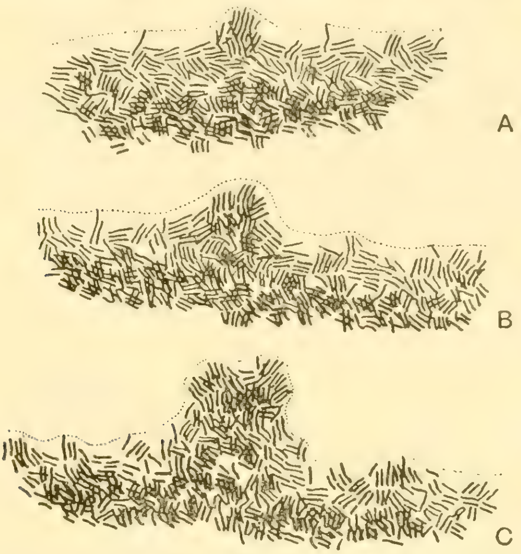


Fig. 1. *Bacillus dendroides.* Successive stages in the formation of a process from a 6 hours old surface colony on nutrient agar.

---

[1] An attempt was made to modify this condition by adding 0·05 per cent. saponin to the medium in order to lower the surface tension of the film. The saponin, however, caused abnormal growth of the organism.

where by their multiplication and further outward movement they further extend the projection. In this manner, the colony is produced into radiating branches (Fig. 2). In a branch, cells can be seen moving toward the outward extremity so that the proximal region of the branch

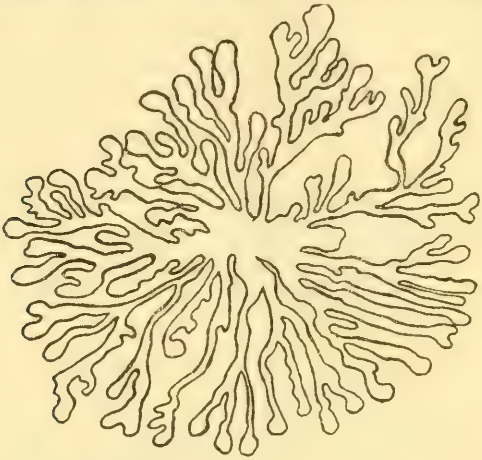


Fig. 2. *B. dendroides.* Young colony, diameter about 1 cm.

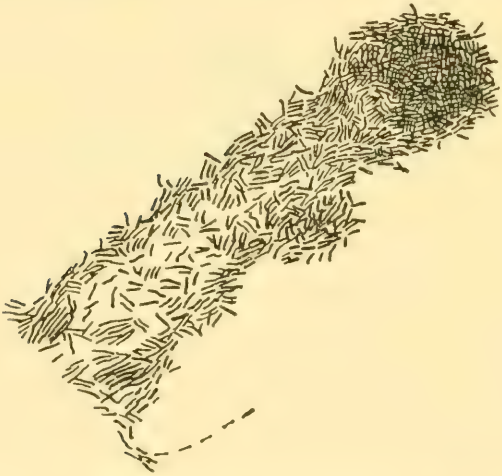


Fig. 3. *B. dendroides.* Young process from a surface colony (nutrient agar 12 hrs. 20° C.).

soon becomes partially depleted of cells, in many places only scattered isolated cells remaining. Towards the outer end the cells are packed close together and near the tip of the process they are piled up, two or more layers of cells overlying one another (Fig. 3). This arrangement can be seen in the living condition and has also been studied in microtome sections of the processes, prepared by the technique developed by Legroux and Magrou (11). It is uncertain whether the outward movement of the cells is due to the repelling influence of substances produced at the centre of the colony or to attraction by some factor in the medium outside.

The branching colony thus produced, spreads over the surface at a surprising rate. Thus, on a nutrient agar plate incubated at 37° C. a colony has been observed to increase in diameter from 0·6 cm. to 1·75 cm. within two hours.

Under conditions favourable to the organism, the spreading growth may continue until the entire surface of the plating is covered with growth. As a rule, however, if the area of a spreading colony be measured at intervals, it is found that a short lag period is followed by rapid spreading expansion which soon becomes increasingly slower, until it finally ceases after a period which varies according to the conditions of growth.

On nutrient agar, the cessation of spreading is accompanied by a change in the morphology of the organisms. Long chains of cells are produced within which endospores soon appear. The formation of these chains of sporangia produces a marked alteration in the appearance of the colony under a low power. At the tips of the branches, the edge is now composed of a mass of convoluted filaments, similar in appearance to those that compose colonies of *Bacillus anthracis*. Growth at the extremity of the branch now takes place laterally, so that the tip expands and becomes club-shaped. In the interior of the colony no further growth occurs, the cells forming endospores.

With the object of discovering a means of checking spreading growth it was important to ascertain the cause which normally brings about the slowing and final cessation of spreading. In the course of many experiments, it has been found that the length of time during which spreading continues is not noticeably affected by the quantity or nature of the food constituents of the medium, although, as will be shown, the amount of spread occurring within that period is greatly influenced by these factors. This suggested that the cause was not nutritional but rather of a physical nature. From our knowledge of the method by which the spreading occurs, through actual motility of the cells in the moisture film, it seemed probable that the cessation of spread was due to the evaporation of this surface moisture. That the drying up of this water film is able to arrest the spreading was shown by the following experiment in which agar platings were dried for various periods before inoculation with the spreading organism. Platings of sterile nutrient agar were poured and were dried for periods of 14, 11, and 2 days respectively and control platings were dried for two hours. Five parallel platings were prepared for each period of drying. The drying took place in an incubator at 30° C. All the platings were then inoculated at the same time with 0·02 c.c. of a suspension of *B. dendroides* spores, and were incubated at 30° C. The growth after 48 hours is recorded in Table III.

Table III.

Spreading of Bacillus dendroides. *Effect of previous drying of plates.*

Period of drying days	Area of growth in 48 hours sq. cm.
14	0·8
11	1·2
2	2·0
Nil	51·6

It will be seen that the spreading is checked by drying of the water film even when this takes place before inoculation. On the plates that had been dried for two or more days, the period of motile spreading was entirely inhibited, and the organism developed long chains of cells in which spores were produced, the colony assuming the anthrax-like edges characteristic of normal growth after spreading has ceased. On the control plates the spreading was quite normal.

Experiments similar to the above have also been carried out, using a "synthetic" agar medium and in this case also the limitation of spreading was observed on platings dried previous to inoculation.

If the retardation and cessation of spreading on platings be normally due to drying of the surface moisture, we should expect that if this drying were prevented, the colony would continue to spread indefinitely, increasing in area at an even rate, without retardation, until the plate was covered. This point was therefore tested by growing *B. dendroides* on platings kept in an atmosphere saturated with moisture.

Six sterile platings of synthetic agar medium were poured and inoculated at the centres with spores of *B. dendroides*. The plates were kept at room temperature, in an atmosphere saturated with moisture in a Novy jar. The areas of growth of the organisms were measured at intervals. Fig. 4 shows the mean areas of growth on the six parallel plates, plotted against the time of incubation. It will be seen that the growth increases in area at a perfectly even rate there being no retardation in the rate of spread even after eleven days.

The result of this experiment also disposes of the view that the retardation of spreading is the result of an increasing accumulation of metabolic products of growth.

It therefore seems clear that the progressive retardation of spreading observed on normal platings is the effect of drying. This drying could operate either by reducing the film of surface moisture in which spreading takes place or by producing an unfavourable increase in the concentration of salts in the medium. This question can be examined by measuring the rapidity of spreading on media in which the concentration of agar is varied. An increase in percentage of the agar will not appreciably increase the concentration of salts, but will reduce the relative amount of free water in the medium and in consequence shorten the time taken for the surface moisture film to evaporate. So that if the mere drying of the surface moisture is the cause, the period of spreading should be shortened as the percentage of agar is increased.

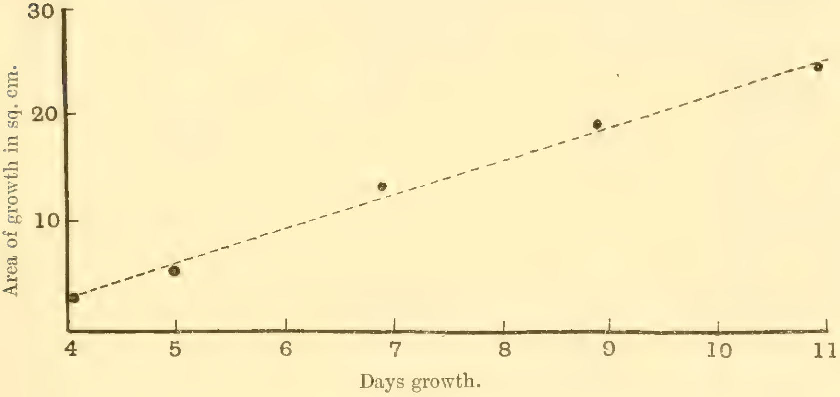


Fig. 4. *B. dendroides.* Spreading over agar plates in a saturated atmosphere.

A "synthetic" agar medium was therefore made up with three percentages of agar—0·5, 1 and 2. Five platings of each medium were poured and after being kept at 20° C. for 24 hours in order to start the drying, were inoculated with *B. dendroides.* The plates were incubated at 20° C., and the area of growth measured at intervals. The mean areas of growth on the sets of five parallel plates are shown in Fig. 5 in which the area of growth is plotted against the time.

It will be seen that with 2 per cent. agar the spreading ceases within five days, while with 1 per cent. agar it continues for a longer period, although the normal retardation of spreading is well shown.

With 0·5 per cent. agar an interesting result appeared. As will be seen from the curve, the increase in colony area took place more slowly, but there was no falling off of the growth, the increase in area taking place quite steadily till the end of the experiment. This steady increase was accompanied by an entire change in the form of growth. The colony was quite circular, nearly transparent and had an indistinct edge, which was not produced into processes. The alteration in the mode and form of colony growth was due to the stiffness of the agar having been reduced to a point at which the motile organisms were able to penetrate the substance of the gel and progress slowly through it. When the growth was examined under a $\frac{1}{6}$-inch objective it was found that instead of forming a layer of surface growth, the rods were distributed throughout the agar, each rod lying separately and moving through the gel with a restricted, jerky motion. The colony grows as an ever-widening disc, since the forces which normally lead to the formation of branches do not operate. Also there is no falling off of the rate of growth, since the

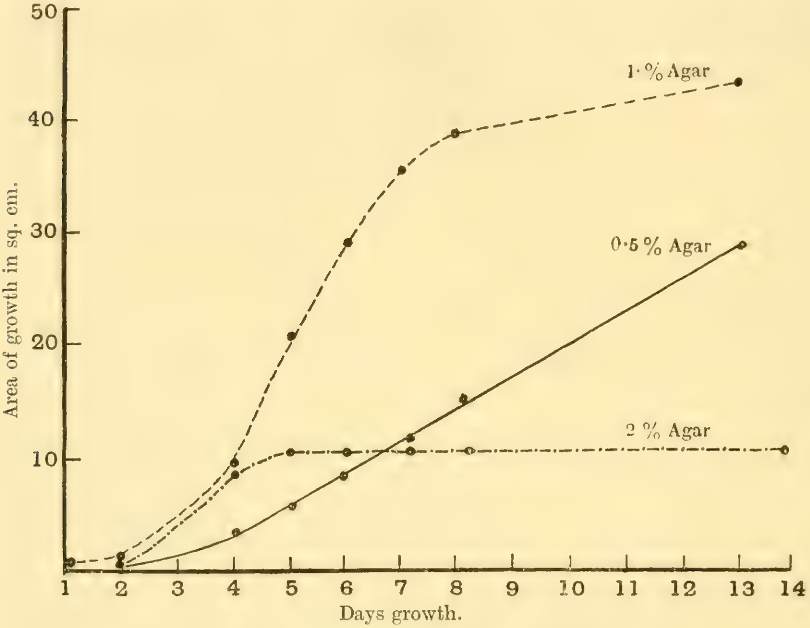


Fig. 5. *B. dendroides*. Spreading growth with varying percentages of agar.

limited motility is not dependent on the surface moisture and the evaporation from the gel itself is too slow to produce visible effect.

The experiment thus shows that slowing and cessation of spreading are due normally to the disappearance of the surface moisture film, for they take place after a longer period when the proportion of water to agar is increased, and do not occur if the gel be so thin as to allow the motile organisms to penetrate away from the surface.

As described above, the cessation of motile spreading on nutrient agar is accompanied by the formation of sporangia and endospores. It appeared possible that the composition of the medium might be so altered as to bring about spore formation and consequent loss of motility and spreading, before the water film dried, thus shortening the period of spreading. I therefore investigated the influence of various substances on spore formation in the organisms. Tests were first made with different organic nitrogen compounds. The medium used as a base in these experiments had the following composition:

K_2HPO_4 ...	1·0 gm.	KNO_3 ...	0·5 gm.
$MgSO_4 . 7H_2O$ ...	0·2 ,,	Dextrose ...	1·0 ,,
$CaCl_2$	0·1 ,,	Agar ...	15·0 ,,
NaCl	0·1 ,,	Water ...	1000 c.c.
$FeCl_3$	0·002 ,,		

To this medium, various nitrogen compounds were added in amounts giving nitrogen equivalent to 0·05 gm. of asparagine. In each medium to be tested, duplicate stab cultures of *B. dendroides* were made and incubated for 14 days at 30° C., after which the growth was examined for spores, both alive and by means of Ziehl Neelsen's spore stain. Media containing the following sources of nitrogen were tested.

1. KNO_3 alone.
2. KNO_3 + Alanine.
3. KNO_3 + Asparagine.
4. KNO_3 + Tyrosine.
5. KNO_3 + Peptone.
6. KNO_3 + Lemco.

On the medium without organic matter and on media 2 and 3, to which alanine and asparagine were added, no spores were produced. On platings of such media, surface colonies cease to spread when the surface water dries off, but the rods retain their flagella and active motility is immediately resumed if the surface be wetted. When, however, the stab cultures had been kept for two months at 30° C., it was found that spore formation had taken place on all media. I also found that if platings of *B. dendroides* on the KNO_3-asparagine medium were dried over H_2SO_4, spores were produced after 11 days. Thus spore formation can take place on these media, but only after much more intense drying than is needed to induce it on nutrient agar. On the media containing peptone, lemco or tyrosine, spores were produced in large numbers within 14 days. It would appear that these substances contain a component which renders spore formation more easily induced. But even on these media, spores were not produced on platings until drying of the surface water had stopped the rapid spread of the organisms. Experiments were also made with various carbohydrates, which, however, were without effect on spore formation. While, therefore, the formation of spores can be retarded by certain conditions of nutrition, it appears on agar platings as a reaction to drying of the surface moisture, and it would seem that it cannot readily be induced until this immediate cause begins to operate[1].

In applying our knowledge of the mode of growth of *B. dendroides*, in an attempt to check its spreading over agar plates, the following facts must be borne in mind.

A. The duration of the period of rapid spreading is limited by the surface moisture of the agar and terminates when this disappears. Methods of drying the agar surface so as to curtail this period do not appear practical in routine work involving a large number of platings. It is not at present possible to shorten the period of spreading by hastening the incidence of spore formation.

B. The rapidity of spreading during the existence of the surface moisture film is influenced by two characters:

(1) The motility of the organism.

(2) Its rate of multiplication.

It has not been found possible to reduce the motility of the organism on platings during this period by any change in the composition of the medium[2]. The rate of multiplication, on the other hand, is greatly influenced by the food supply, and it seemed probable that by checking this during the period of spreading, the area of spread could be much reduced.

It is known that the content of organic matter of the medium influences the formation of spreading colonies on plates (3). I therefore decided to investigate the influence of the organic nitrogen constituent of the medium on the rate of multiplication of *B. dendroides*, comparing its growth on media containing, respectively, peptone, "lemco," and a pure amino-acid.

---

[1] Cultural conditions liable to inhibit the development of other soil organisms on the plates are not here considered.

[2] H. Braun (16) found that *B. proteus*, if grown on agar media in which the nutrient material and salts were reduced to $\frac{1}{10}$ the normal concentration, lost its flagella and consequently formed non-spreading colonies. Unfavourable food conditions, however, do not appear to influence the motility of *B. dendroides*, though affecting its multiplication rate.

The medium used as a basis had the following composition:

Medium CV.

K_2HPO_4	1 gm.	$FeCl_3$	0·002 gm.
$MgSO_4 . 7H_2O$	0·2 ,,	KNO_3	0·5 ,,
$CaCl_2$	0·1 ,,	Mannitol	1·0 ,,
NaCl	0·1 ,,	Water	1000 c.c.

To the above medium the organic nitrogen supply was added in an amount giving nitrogen approximately equivalent to 0·05 per cent. asparagine. The reaction of the media was standardised to pH 7·4, immediately prior to autoclaving. Duplicate tubes containing 10 c.c. of medium were inoculated with 0·5 c.c. of a suspension of a young agar culture of *B. dendroides*. The tubes were incubated at 30° C., and, at intervals of 12 hours, the number of organisms per c.c. was estimated from counts made in a Thoma counting chamber, the mean count of the duplicate tubes being taken in each case.

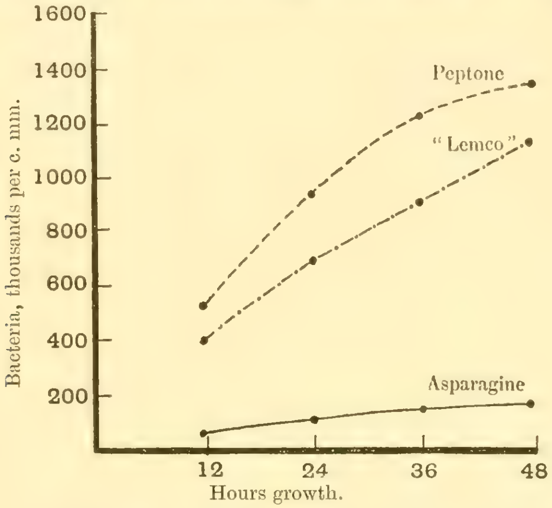


Fig. 6. *B. dendroides*. Effect of the organic nitrogen source on multiplication.

The curves, Fig. 6, show the multiplication of the organisms in media containing "lemco," peptone, and asparagine.

It will be seen that, with asparagine, the multiplication is very markedly less rapid than in the presence of peptone or meat extract.

It therefore appeared probable that the amount of spreading would be correspondingly decreased on a medium containing amino-acid as the source of organic nitrogen. I therefore measured that increase in area of surface growths of *B. dendroides* upon the media employed in the last experiment, made up with 1·5 per cent. agar. A medium was also tested containing equivalent nitrogen in the form of tyrosine. Tubes of each medium were autoclaved and poured into sterile petri dishes. Each plate, when the agar had set, was inoculated at the centre with 0·01 c.c. of a suspension of a 48-hour old culture of *B. dendroides*. The plates were incubated at 20° C., and the area of growth on each plate was measured at intervals, over squared paper. Eight parallel platings of each medium were prepared and the mean areas of growth are shown in Fig. 7, in which the area of growth is plotted against the time.

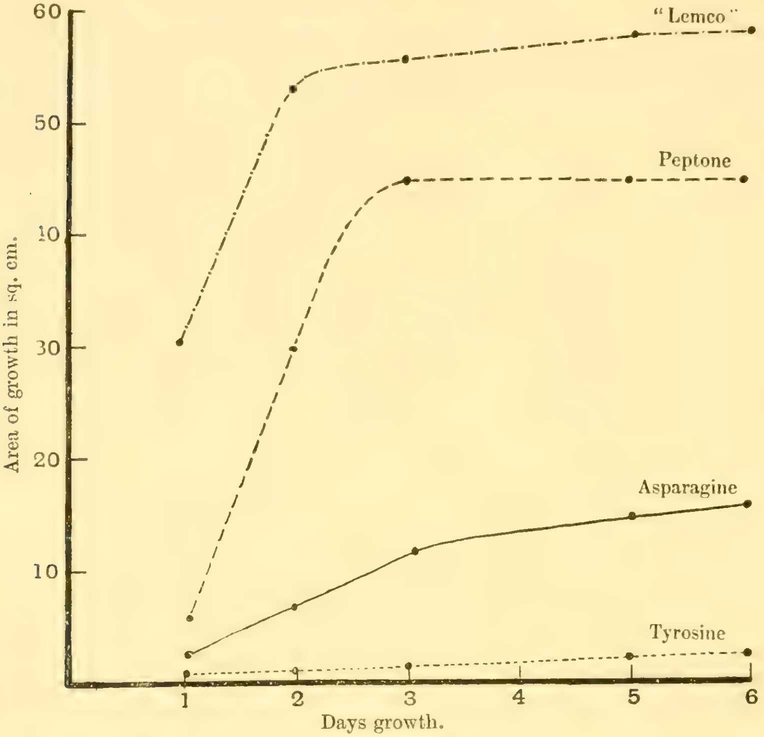


Fig. 7. *B. dendroides.* Effect of the source of organic nitrogen on spreading growth.

The curves show that spreading growth is very much reduced when peptone or meat extract is replaced in the medium by a simple amino-acid. This explains the fact, observed by Conn (3), that on Lipman and Brown's peptone agar, "overgrowths are often so abundant...as to interfere with counting and prevent the isolation of pure cultures from the colonies," and also that these overgrowths are reduced on his medium containing ammonium phosphate and sodium asparaginate.

On the tyrosine medium scarcely any growth took place. It appears that this is due, not to any inhibiting action of the tyrosine, but to the inability of the organism to make full use of the tyrosine molecule in its nutrition. This is shown in the following experiment, in which the growth was compared on media similar to those tested in the last experiment but having organic nitrogen supplied as follows:

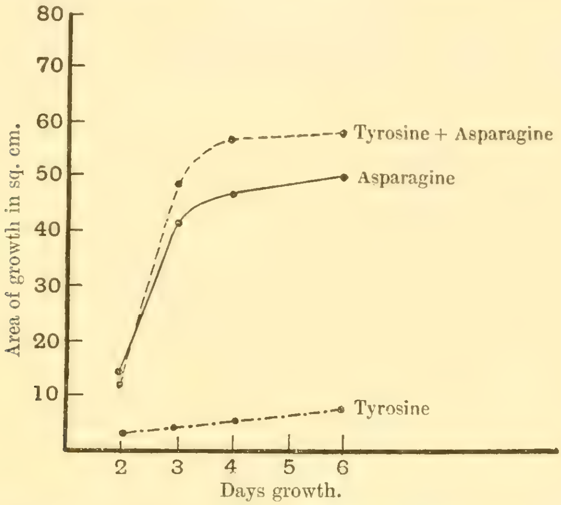


Fig. 8. *B. dendroides.* Effect on tyrosine and asparagine on the spreading growth.

A. 0·12 per cent. tyrosine.
B. 0·12 per cent. tyrosine + 0·05 per cent. asparagine.
C. 0·05 per cent. asparagine.

The test was conducted in a manner similar to the last experiment except that the plates were incubated at 25° C. to accelerate the growth.

The curves (Fig. 8) show the mean areas of growth, measured at intervals, of ten parallel platings in the case of media B and C and of seven parallel platings in the case of medium A. It will be seen that in the presence of asparagine, tyrosine does not check but slightly stimulates the growth, whereas with tyrosine alone the growth is very slight. Tests made with the tyrosine medium, however, showed that it was unsuitable for use in bacterial count work, owing to the very rapid development of moulds which took place on it.

Tests were also made with media containing glycocoll and alanine. These media were found to give results comparable with but not better than those obtainable with asparagine.

The entire omission of organic nitrogen was found greatly to reduce the spreading, but the number of bacterial colonies which developed from a suspension of Rothamsted soil, when plated on such a medium, was so much reduced by the omission of organic nitrogen, that the medium was unsuitable for counting work.

It was therefore decided to use asparagine as the organic nitrogen supply in the medium, and trials were made to ascertain the concentration which produced the least spreading of *B. dendroides*, while allowing the best colony development when the medium was used for counting other bacteria. In these trials medium CV (p. 259) made up with 1·5 per cent. agar was used. The technique used to estimate the spreading was similar to that described above. Fig. 9 shows the growth area of *B. dendroides* with 0·1 per cent. and 0·05 per cent. asparagine. The colony development on these two media was also tested, eight parallel platings of a suspension of Broadbalk soil being made on each medium. The colony counts obtained were significantly higher on the medium containing 0·05 per cent. asparagine.

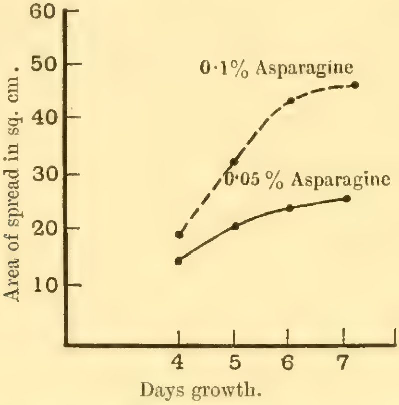


Fig. 9. *B. dendroides.* Effect of asparagine content on spreading.

The increase in the asparagine content is thus detrimental as it both favours the spreading of *B. dendroides* and is harmful to the development of other organisms.

The effect of reducing the asparagine content below 0·05 per cent. is shown in Fig. 10, where the increases in area of surface growths of *B. dendroides* on media containing 0·05 per cent., 0·005 per cent. asparagine and no asparagine, are plotted. It will be seen that while the growth is reduced in the total absence of organic nitrogen, the reduction of asparagine content to 0·005 per cent. was without appreciable influence on the spreading.

This reduction, however, was found to produce a significant falling off in the number of colonies developing when a suspension of Rothamsted soil was plated on the two media.

The optimum concentration of asparagine in the count medium is therefore in the region of 0·05 per cent. This concentration has been adopted in the medium.

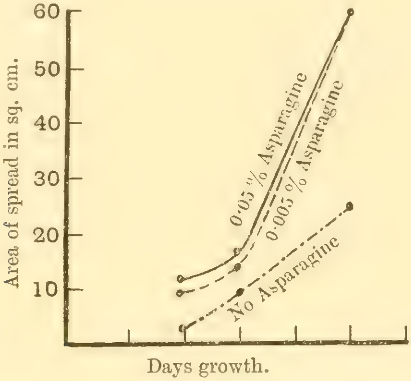


Fig. 10. *B. dendroides.* Effect on asparagine content of medium on spreading.

The additional nitrogen in the medium was supplied as KNO_3 and the effect of this compound on the spreading was therefore tested. The medium used in this and the next experiment had the following composition, and to it KNO_3 and asparagine were added in the amounts indicated.

K_2HPO_4 ...	1·0 gm.	$CaCl_2$ ...	0·1 gm.
$MgSO_4 . 7H_2O$ ...	0·2 ,,	$FeCl_3$ ...	0·002 ,,
NaCl	0·1 ,,	Mannitol ...	1·0 ,,
Agar	15·0 ,,	Water ...	1000 c.c.

Table IV shows the areas of spreading growth of *B. dendroides* after two and eight days incubation at 20° C. on media nitrogen supply as shown.

Table IV.

Effect of Nitrate and Asparagine on the spreading of Bacillus dendroides.

Medium	Source of nitrogen	Area of spread of *B. dendroides* in 2 days sq. cm.	Area of spread of *B. dendroides* in 4 days sq. cm.
A.	0·05 % asparagine	3·3	56·5
B.	0·05 % asparagine 0·05 % KNO_3	2·8	58·6
C.	0·05 % KNO_3	0·73	12·6
D.	0·1 % KNO_3	0·58	13·4

It will be seen that complete omission of KNO_3 in medium A is without effect on the spreading, though the omission of asparagine checks the spreading even where the KNO_3 is increased to 0·1 per cent. A reduction in the KNO_3 content below 0·05 per cent. is therefore of no assistance in reducing "spreading."

The effect of a higher concentration of KNO_3 was next tried. Two media were compared, having nitrogen supplied as follows:

A. 0·2 per cent. KNO_3 0·05 per cent. asparagine.

B. 0·05 per cent. KNO_3 0·05 per cent. asparagine.

Fig. 11 shows the increase in area of surface growth of *B. dendroides* on these two media, each point on the curve representing the mean of twenty parallel plates.

It will be seen that in the presence of asparagine, the higher concentration of KNO_3 increases the spreading on platings. A suspension of Barnfield soil was plated on the above media, and on one containing 0·1 per cent. KNO_3 + 0·05 per cent. asparagine, and no significant difference could be found in the number of colonies developing on the three media. There is thus no advantage in lowering the concentration of KNO_3 below 0·05 per cent. while a higher concentration tends to stimulate spreading. This percentage was therefore adopted for use in the medium.

The additional source of energy in the medium was supplied as mannitol. This compound was used in preference to a sugar for reasons, dealt with below, connected with the change in reaction during sterilisation. Experiments on the effect of varying percentages of mannitol on the spreading of *B. dendroides* were made. It was found that an increase in the mannitol content from 0·05 per cent. to 0·1 per cent. did not stimulate spreading but that a further increase of 0·2 per cent. caused slightly more spreading growth. Counts were also made of the number of colonies which developed from a single diluted suspension of Rothamsted soil on media containing 0·05 per cent., 0·1 per cent. and 0·2 per cent. mannitol. Eight parallel platings of each medium were poured and incubated for seven days at 20° C. No significant differences in the number of colonies, on the different media, were found. It was decided to include 0·1 per cent. mannitol in the medium as this gives the maximum energy supply without increasing "spreading."

Attention was also turned to the effect of phosphate supply on the spreading of *B. dendroides*. The increase in area of surface growth of this organism was measured on media containing 0·2 per cent., 0·1 per cent., 0·05 per cent. and 0·025 per cent. K_2HPO_4 respectively. Variation of the concentration of K_2HPO_4 between these limits was found to be without significant influence on the "spreading."

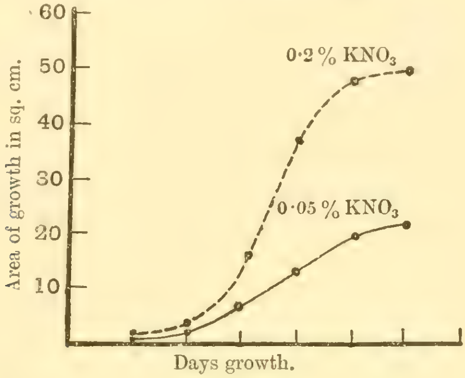


Fig. 11. *B. dendroides*. Effect of nitrate content of medium on spreading.

3. MAINTENANCE OF STANDARD REACTION.

One of the features necessary in a medium to be used in quantitative work is that its reaction should not vary sufficiently to affect the colony development.

The reaction is commonly standardised just before sterilisation. It is during sterilisation that changes occur which are not always constant, so that uniformity in the sterile medium may be lost. In developing a medium, it is therefore important to consider the change in hydrogen ion concentration which will occur during sterilisation. If possible, the medium should be so constituted that this change shall not be sufficient to affect colony development. In this portion of the work, therefore, it was necessary, firstly, to ascertain the limits of change in reaction which might occur in the medium without affecting the number of colonies that developed, and secondly, to develop a medium whose change of reaction during sterilisation would not reach this limit.

In the measurements of H-ion concentration involved in this work, the indicator method developed by Gillespie(12) was used. This method depends on the assumption that, at any given H-ion concentration, a definite percentage of the indicator is in the acid form and the remainder in the alkaline form. If it is known what these proportions are for a given indicator at a given reaction, the colour shown by the indicator in a solution of this reaction, can be imitated by dividing the indicator in the correct proportions between two solutions, one of which contains excess of acid and the other excess of alkali. These ratios have been ascertained by Gillespie for a number of indicators over a range of *p*H values. Colour standards prepared by this method consist of pairs of tubes, one containing dilute acid and the other dilute alkali. Each pair together contains ten drops of the indicator, these drops being divided between the two tubes according to the ratio ascertained for the *p*H value required.

Before employing this technique, it was thought advisable to test the accuracy of readings obtained with it, as compared with the method of Clark and Lubs(13) in which the indicator colour standards are made up in standard buffer solutions of definite *p*H value. These tests of Gillespie's method were carried out at Rothamsted by Mr E. A. Fisher, to whom, also, I am indebted for much help and advice throughout the work connected with the reaction of the medium. The following indicators were tested:

	Range
Brom Cresol Purple (Dibromo-o-cresolsulphonphthalein)	*p*H 5·6–*p*H 6·8
Brom Thymol Blue (Dibromo-thymolsulphonphthalein)	*p*H 6·5–*p*H 7·7
Phenol Red (Phenolsulphonphthalein)	*p*H 7·2–*p*H 8·3

With each indicator, seven pairs of tubes were made up as colour standards, as described by Gillespie. The indicators were added in drop ratios ranging from 8 acid:2 alkaline, to 2 acid:8 alkaline. The colour of each pair of tubes was compared with a series of colour standard tubes prepared by Clark and Lubs' method in each of which ten drops of indicator were added to 10 c.c. of a buffer solution of known H-ion concentration.

The readings thus obtained are shown in Fig. 12, in which the actual readings are plotted on "smoothed" curves. In those cases where the actual readings lay off the smoothed curves, the pairs of drop ratio tubes were made up several times and invariably gave similar readings. It is therefore believed that these irregularities were due to slight errors in the buffer solution standards. Where they differ from ours the "smoothed" curve readings, given by Gillespie, are plotted in broken line beside our readings. It will be seen that the two series agree closely in the case of Brom Thymol Blue, but that there is a constant disagreement of about 0·05 *p*H on the alkaline side in the case of Phenol Red and of about 0·1 *p*H on the acid side, with Brom Cresol Purple. It is considered probable that this is due to slight differences in the samples of indicator used in the two cases.

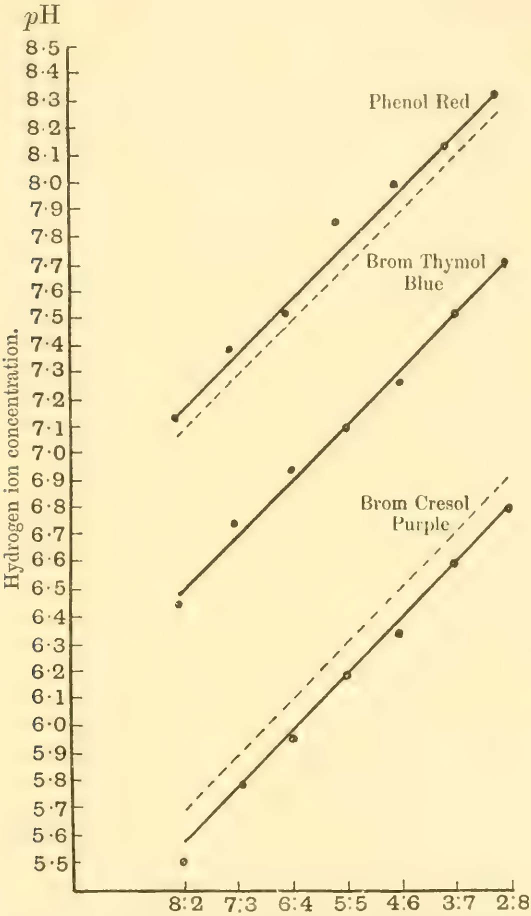


Fig. 12. Drop ratios. (Acid indicator: alkaline indicator.)

The smoothed curves thus experimentally obtained were used in making the readings of *p*H value in the work described below.

In investigating the effect of the H-ion concentration of the substratum on the number of colonies that developed thereon a medium of the following composition was employed:

K_2HPO_4 ...	1·0 gm.	KNO_3 ...	0·5 gm.
$MgSO_4.7H_2O$ ...	0·2 ,,	Asparagine ...	0·5 ,,
NaCl	0·1 ,,	Mannitol ...	1·0 ,,
$CaCl_2$	0·1 ,,	Agar ...	1·5 ,,
$FeCl_3$	0·002 ,,	Distilled water	1000 c.c.

The medium was filtered at 100° C. and divided into 200 c.c. portions which were sterilised at 15 lbs. pressure for 15 minutes in the autoclave.

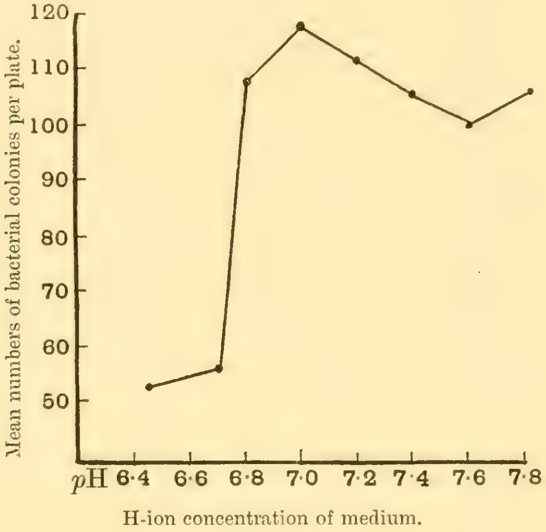


Fig. 13. Effect of the reaction of the medium on colony development.

The H-ion concentrations of the media were then adjusted to values ranging from pH 6·45 to pH 7·8, with sterile $N/10$ HCl and $N/10$ NaOH, using aseptic technique. A single diluted suspension of Rothamsted soil was plated on each medium, six to eight parallel platings being made in each case. The colonies were counted after 10 days incubation at 20° C. In Fig. 13 the mean number of colonies per plate on each medium is plotted against the pH value. The differences in colony development on media ranging from pH 6·8 to pH 7·8 are barely significant, having regard to the variance between parallel platings, there being, however, some indication of an optimum reaction near neutrality. On the acid side, however, there is a remarkable fall in colony development on media having an acidity higher than *p*H 6·8.

Thus, in preparing a standard medium, some latitude for changes in reaction during sterilisation is permissible, if its reaction be kept within a range of from *p*H 7 to *p*H 7·8. On the other hand, if the medium be on the acid side of neutrality, a slight increase in its acidity may cause its H-ion concentration to reach the critical point involving a marked fall in colony development.

For this reason, the use of an ammonium salt in a standard medium is a disadvantage, for if such a medium be brought to the alkaline side of neutrality with NaOH, some of the ammonia is liberated, and, during sterilisation, is driven off, bringing the reaction back to neutral point. A slight hydrolysis of the carbohydrate constituent of the medium during sterilisation is now sufficient to bring the H-ion concentration up to a dangerous point. Thus if Conn's sodium asparaginate agar(3) (containing ammonium phosphate) be adjusted to a slightly alkaline reaction before sterilisation, the H-ion concentration of the medium after autoclaving is found to be approximately *p*H 6·8, so that a slight further increase in acidity, arising from any cause, would harmfully affect the medium.

From these considerations, it was decided to use nitrate as the inorganic nitrogen source in the medium. Trials showed that the colony development was as good on a neutral medium containing nitrate as on one containing an ammonium salt, while, in the former case, the risk of a detrimental increase in acidity, during autoclaving, need not be incurred. The following comparison shows the advantage of nitrate in this connection. The medium CV (see p. 259) was made up with 0·05 per cent. asparagine, and divided into two portions to one of which 0·1 per cent. $(NH_4)_2SO_4$ was added, and to the other equivalent nitrogen in the form of KNO_3. The reaction of both media was standardised to *p*H 7·4 and the media were autoclaved at 15 lbs. pressure for 15 minutes. After sterilisation the reaction of the ammonium sulphate medium was found to be *p*H 6·7, while that of the nitrate medium was *p*H 7·2.

The percentage of KNO_3 used in the count medium was finally fixed at 0·05 per cent. as a result of work above described on the control of spreading colonies.

The chief cause of the development of acidity in media during sterilisation is believed to be the hydrolysis of the carbohydrate constituent. I therefore made tests of media containing various sugars and related compounds to discover which source of energy material was most suitable.

The following medium was used as a basis in these tests:

K_2HPO_4 ...	1·0 gm.	KNO_3 ...	0·5 gm.
$MgSO_4.7H_2O$ ...	0·2 ,,	Asparagine	0·5 ,,
CaCl	0·1 ,,	Agar ...	15·0 ,,
NaCl	0·1 ,,	Water ...	1000 c.c.
$FeCl_3$	0·002 ,,		

This medium was divided into five portions to which were added the following compounds in 0·1 per cent. concentration:

A. Dextrose. B. Saccharose. C. Mannitol. D. Lactose. E. Glycerine.

The media were adjusted to a H-ion concentration of *p*H 7·05 immediately before sterilisation for 15 minutes at 15 lbs. pressure. Directly after autoclaving, the reaction was again measured. Six parallel platings of a diluted suspension of Rothamsted soil were made on each medium. Table V shows the changes of reaction during sterilisation and the mean number of colonies per plate with each medium. Table VI shows the results of another similar experiment in which glucose, saccharose and mannitol media were compared.

Table V.

Change in Reaction of Media on Sterilisation.

Energy material	*p*H value before sterilisation adjusted to	*p*H value after sterilisation	No. of colonies on each plate	Mean no. of colonies
Dextrose	7·05	6·7	13, 10, 13, 18, 15, 14	13·8
Saccharose	7·05	6·8	19, 18, 16, 11, 10, 10	14·0
Mannitol	7·05	6·9	19, 18, 17, 16, 13, 10	15·5
Lactose	7·05	6·7	13, 12, 12, 11, 10, 10	11·3
Glycerine	7·05	6·75	18, 13, 12, Sp. Sp. Sp.	14·3

Table VI.

Change in Reaction of Media on Sterilisation.

Energy material	*p*H value before sterilisation	*p*H value after sterilisation	No. of colonies on each plate	Mean no. of colonies
Dextrose	7·2	6·7	14, 21, 13, 14, 14, 13, 14, 13	14·5
Saccharose	7·2	6·7	19, 17, 17, 16, 15, 14, 13	15·8
Mannitol	7·2	6·95	19, 19, 22, 22, 18, 17, 17, 17	18·9

It will be seen that the medium containing mannitol changes least in reaction during sterilisation and at the same time gives a good colony development. With lactose, the number of colonies was somewhat reduced and the individual colonies were dwarfed. With glycerine, marked spreading of *B. dendroides* occurred on the plates. Mannitol was consequently adopted as the energy source, in addition to the asparagine, in the count medium, and subsequent work has shown its advantage, both on account of the slight change in reaction produced by it on autoclaving, and of the good development of colonies on the medium.

4. Preparation of the Medium.

On account of the changes and interactions which take place in a nutrient medium in the course of its preparation, it is necessary, in order to obtain uniform results with it, that the method of preparation should be carefully standardised. The need for this precaution is well shown by the effect of variations in the method of filtration, discussed below.

The medium here developed has the following composition:

K_2HPO_4 ...	1·0 gm.	KNO_3 ...	0·5 gm.
$MgSO_4 + 7H_2O$	0·2 ,,	Asparagine	0·5 ,,
$CaCl_2$	0·1 ,,	Mannitol ...	1·0 ,,
NaCl	0·1 ,,	Agar ...	15·0 ,,
$FeCl_3$	0·002 ,,	Water to	1000 c.c.

In making up this medium, the following technique was finally adopted. The phosphate, nitrate and asparagine are dissolved in the distilled water and the $MgSO_4$, $CaCl_2$, NaCl and $FeCl_3$ added from standard solutions, in the order named. The agar is then added and dissolved at 100° C. The medium is then filtered at this temperature, by being passed twice through a layer of absorbent cotton-wool half an inch thick. The mannitol is then dissolved in the filtrate. It is then allowed to cool to 60° C. and its reaction adjusted against Brom Thymol Blue to pH 7·4. The medium is then poured into tubes and sterilised at 15 lbs. pressure for 15 minutes.

In the earliest work done with this medium, some differences were found between different batches of medium. These differences were eventually traced to variations in the temperature at which filtration was carried out. The effect of the temperature of filtration is shown in the following experiment. Three litres of the medium were made up and divided into two portions, one of which (A) was filtered through cotton-wool at 100° C. and the other (B) at 50° C. A single diluted suspension of Rothamsted soil was plated on the two media and the mean number of colonies on ten parallel platings on each medium was taken. The results are shown in Table VII, and indicate a perceptible fall in the nutritive value of the medium when filtered at the lower temperature. (See also Fisher, Thornton and Mackenzie(2).)

Table VII.

	Medium filtered at 100° C.	Medium filtered at 50° C.
Colonies on each plate	68	49
	65	49
	65	48
	58	48
	57	47
	54	45
	53	44
	53	41
	51	39
	41	39
Mean	56·5	44·9

Tests were also made as to the comparative advantages of filtration through filter paper and cotton-wool. No advantage was found in the former method, either with reference to the total number of colonies developing, or to the uniformity between batches of medium separately filtered.

Length and Temperature of Incubation. In working with this medium, the best results have been obtained by incubating the platings at 20° C. for 10 to 12 days. In a shorter period the slow-growing colonies have either not developed or are very small. These results agree with the finding of Cunningham (14).

5. Tests of the Count Medium.

There are two respects in which a medium for use in quantitative work should display uniformity. In the first place, it must be reproducible, that is, different batches of medium should be similar in the results obtained with them. Secondly, parallel platings of a suspension of soil, made with a single batch of medium, should develop the same number of colonies within the limits of random sampling variance. Uniformity in this latter respect will depend mainly upon limitation of the growth of fast growing organisms and especially of moulds and bacteria that form spreading colonies or develop toxic products, whose chance appearance on platings may affect the number of colonies developing thereon. These two aspects of the medium must be separately tested.

The capacity for colony development on the present medium has been found to be closely reproducible in different batches, if the method of preparation be carefully standardised. In the following test, five batches of medium were separately prepared, and a single suspension of

Rothamsted soil plated on them, eight parallel platings being made of each medium. Table VIII shows the colonies developing on each plate. It will be seen that no significant differences are shown between the different batches.

Table VIII.

Colony Development from a Single Soil Suspension, Plated on five different Batches of Count Medium.

	Medium A	Medium B	Medium C	Medium D	Medium E
Number of colonies on each plate	92	88	88	82	85
	84	85	88	82	84
	80	78	82	80	80
	77	78	80	76	76
	76	76	78	74	73
	75	75	76	70	70
	75	75	75	70	67
	72	70	75	68	Sp.
Mean no. colonies per plate	78·87	78·12	80·25	75·25	76·43

The uniformity between parallel platings on the same batch of medium has been studied from about 3000 platings made in the Protozoology and Bacteriology Departments at Rothamsted. A statistical analysis of this mass of data has been made by Mr R. A. Fisher (Fisher, Thornton and Mackenzie(2)). The results, which are on the whole quite satisfactory, are published separately.

About 4000 platings have been made on the medium since its development. In the majority Rothamsted soil was used, but 240 of the platings were of a light ironstone soil from Kingsthorpe, Northamptonshire. Although "spreading" organisms occur on about 40 per cent. of the platings of Rothamsted soil, only some 3 per cent. of the platings were lost owing to the development of spreading colonies over the surface.

6. SUMMARY.

1. For bacterial count work the first essential in a medium is that it should be uniform and reproducible in its results.

2. In the medium here described, details of which are given on p. 271, reproducibility has been achieved by the use of pure chemical compounds in an agar medium and by selection of such constituents as will not produce a significant change of reaction during sterilisation.

3. On agar media, surface spreading colonies interfere with the accuracy of the results. A special study was made of a common spreading

organism, *B. dendroides*. This organism spreads by active motility, and the factors controlling its spread were found to be (1) the existence of a surface film of water on the agar, and (2) the rate of multiplication previous to the drying of this film. A medium was developed on which this rate of multiplication was greatly reduced and on which, consequently, spreading is greatly restricted.

4. Tests of the medium have shown that the results obtained with it are uniform and can be reproduced in different batches of medium.

REFERENCES.

(1) Breed, R. S. and Stocking, W. A. (1920). The accuracy of bacterial counts from milk samples. New York Agricultural Experiment Station. *Technical Bulletin*, No. 75.

(2) Fisher, R. A., Thornton, H. G. and Mackenzie, W. A. (1922). The accuracy of the plating method of estimating the density of bacterial populations, with particular reference to the use of Thornton's agar medium with soil samples. *Annals of Applied Biology*, IX.

(3) Conn, H. J. (1914). Culture media for use in the plate method of counting soil bacteria. New York Agricultural Experiment Station. *Technical Bulletin*, No. 38.

(4) Fischer, H. (1910). Zur Methodik der Bakterienzahlung. *Centralblatt für Bakteriologie*, Abt. II. Bd. 25, p. 457.

(5) Temple, J. C. (1911). Influence of stall manure upon the bacterial flora of the soil. *Georgia Experiment Station Bulletin*, 95.

(6) Lipman, J. C. and Brown, P. E. (1910). Media for the quantitative estimation of soil bacteria. *Centralblatt für Bakteriologie*, Abt. II. Bd. 25, p. 447.

(7) Fellers, C. R. (1916). Some bacteriological studies on agar-agar. *Soil Science*, II. 255.

(8) Cunningham, J. (1919). Note on the preparation of purified agar powder with increased powers of filtration. *Indian Journal of Medical Research*, VI. 560.

(9) Holzmuller, K. (1909). Die Gruppe des *Bacillus mycoides* Flugge. *Centralblatt für Bakteriologie*, Abt. II. Bd. 23, p. 304.

(10) Hill, H. W. (1902). "Hanging Block" preparations foı the microscopical observation of developing bacteria. *Journal of Medical Research*, VII. 202.

(11) Legroux, R. et Magrou, J. (1920). État organisé des colonies bactériennes. *Annales de l'Institut Pasteur*, XXXIV. 417.

(12) Gillespie, L. J. (1920). The Colorimetric determination of Hydrogen ion concentration without Buffer mixtures, with especial reference to soils. *Soil Science*, IX. 115.

(13) Clark, W. M. (1920). *The determination of Hydrogen ions.* Baltimore.

(14) Cunningham, A. (1913). A note on the plate method for enumeration of Bacteria. *Journal of Hygiene*, XIII. 433.

(15) Noyes, H. A. and Grounds, G. L. (1918). Number of colonies for a satisfactory soil plate. *Proceedings Indiana Acad. Science*, p. 93.

(16) Braun, H. (1921). Ueber die Wirkung der Unternahrung auf Bakterien. *Zeitschrift für allgemeine Physiologie*, Bd. 19, p. 1.

(*Received July* 21*st*, 1922.)

STUDIES ON THE APPLE CANKER FUNGUS

II. CANKER INFECTION OF APPLE TREES THROUGH SCAB WOUNDS[1]

By S. P. WILTSHIRE, B.A., B.Sc.

(*University of Bristol Agricultural and Horticultural Research Station, Long Ashton.*)

(With Plate XII.)

INTRODUCTION.

In a previous paper (1) reference was made to the fact that the canker fungus *Nectria galligena*, Bres., can enter the apple tree through the wounds caused by the scab fungus *Venturia inaequalis*. It is the purpose of this paper to describe this process in detail.

SYMPTOMS.

The scab fungus infects the shoots of susceptible varieties of apples during the autumn and winter following their growth, the first infections usually being found before the trees defoliate. In the spring most of the pustules are surrounded by a cork layer and are subsequently completely excluded from the tree, the only trace of the infection finally being a slight roughness of the bark.

Sometimes, however, this course of events is disturbed. The cortex round the small scab pustule shows signs of blackening, and this is accompanied in some cases by a swelling of the bark due to the growth of the tissues beneath the infection (see Pl. XII, fig. 1). Very early stages, in which the discoloration is extremely slight, can sometimes be identified. When the canker fungus has once got in (for as will be seen later this difference from the normal development is due to *Nectria galligena*, Bres.) it usually develops so rapidly that an area about 5 mm. in diameter is completely killed and blackened before any attempt at phellogen formation becomes effective. The canker area is usually somewhat sunken, there is no crack in the bark between the healthy and diseased tissue and the little scab infection can often be identified in the middle of the scar. In the autumn and winter canker infections of scab wounds are thus most frequently found in this stage (see Fig. 2). Later stages of development are often characterised by the formation of well-defined cracks at the edge of the infected area and a slight swelling of the adjacent tissue (see Figs. 3 and 4). If the tree is sufficiently vigorous to form a cork layer round such a scar before the wood has become infected, the canker makes very little progress, and the tree makes a good fight against the fungus. Often, however, the whole of the cortex becomes infected and the fungus reaches the woody tissues. In these cases, examples of which are seen in Figs. 5 and 6, the scar is more like a normal canker produced by the canker fungus. It is somewhat difficult to assign any particular method of infection to a mature canker. The presence of the concentric cracks in the bark, however, localises the original point of infection and the appearance of this spot is sometimes strongly suggestive of infection through a scab wound. Fig. 7 is a photograph of such a case and other instances have been found on the pear as well as the apple. Fructifications of the fungus are not borne until the canker is well developed, but on keeping young infections in a moist chamber for two or three days, a few small conidial pustules generally appear and afford evidence of the presence of the canker fungus.

---

[1] A grant in aid of publication has been made for this communication.

The occurrence of this type of infection has not been found to be nearly so common as that of the leaf scar infection previously described but it is probably as prevalent as the infection which takes place through woolly aphis galls. In some years when the autumn has been specially damp, the shoots of the previous winter are often found to be killed off in large numbers. Such shoots are usually heavily infected with scab and although they frequently bear leaf scar infections of the canker fungus, it is probable that canker infections of scab wounds are responsible for a good proportion of the damage.

MICROSCOPIC DETAILS.

The establishment of the canker mycelium upon the scab stroma. Early in the autumn, when the scab pustules are very small and before any really definite signs of infection can be observed by the naked eye, microscopical examination has revealed the presence of *Nectria galligena* in a number of instances. The minute cracks which occur in the bark covering the young scab infection afford a favourable lodgment for the conidia of the canker fungus, which germinate readily under the moist conditions usually prevailing in the autumn. The mycelium thus produced proceeds to establish itself on the stroma of the scab fungus. This done, it begins to form typical conidia, which afford proof of the identity of the canker fungus. Fig. 8 shows a drawing of a small pustule of the canker fungus growing just over a young scab infection. The material concerned was freshly collected from the plantation and the conidia illustrated had developed there under natural conditions. A section through a young scab infection attacked by *Nectria galligena* which has reached a slightly later stage is shown in Fig. 9, the central portion of which is enlarged in Fig. 10. Here the scab fungus has burst open the bark and the canker fungus has attacked the mycelium of *Venturia inaequalis* and produced numerous conidia. It is rather difficult to distinguish between the mycelia of the two fungi, but generally speaking in a mixed stroma, the mycelium of the scab fungus appears dark and somewhat thick-walled whilst that of the canker fungus is hyaline, less distinct and not so robust. Its cells, too, are rather smaller in size than those of the scab fungus. The two mycelia are not absolutely intermingled but are naturally divided into areas. In Fig. 10 the areas (*a*) and (*b*) are probably *Nectria* mycelium whilst the areas (*c*) and (*d*) are probably scab.

The penetration of Nectria galligena *from the scab stroma into the cortex.* As soon as the canker fungus has gained a firm hold on a scab pustule, a struggle between host and parasite commences. It has been shown (2) that if a cut is made in the cortex of an apple tree in such a way that it does not extend to the wood, and if conidia or hyphae of *Nectria galligena* are placed on such a wound, the host merely forms a cork layer round the portion of cortex which becomes infected and the fungus fails to establish itself. The experiments were chiefly carried out in the summer when the trees were active, and the results were so positive that for some time it appeared very difficult to understand how the canker fungus could infect from a shallow injury such as that effected by the scab fungus. It is indeed probable that, in some cases at any rate, such a cork layer is effective in preventing the development of canker even in cases of *Nectria* infection of scab pustules. In Fig. 9 the whole of the infected portion is surrounded by a cork layer formed some distance below the original seat of the trouble. If such a layer became matured before the *Nectria* reached it, then it is extremely probable that the latter could progress no further, unless assisted by a fresh development of the scab fungus.

With the formation of a phellogen, the cortical cells below frequently begin to divide to produce new tissue in addition to that arising from the activity of the phellogen. The result of this growth is that cracks sometimes occur in the bark in the vicinity of the infected portion (see Fig. 9); but although mycelium is sometimes found in such places, its occurrence is not frequent enough to suggest that infection by *Nectria* is secured by this means.

The cork layer, however, is not always developed quickly enough to confine the canker fungus to the outside of the barrier. The scab fungus is able to penetrate suberised tissue and its normal procedure is simply to grow through any cork layer formed below it, especially at the edges of the infected region. *Nectria* appears to follow the *Venturia* to some extent. The antagonism which might be expected to be exerted by the scab fungus appears to be quickly overcome and *Nectria* subsequently dominates the situation. Its hyphae begin to grow inwards between the cells of the cortical tissue, which towards the outside of the stem, includes very few intercellular spaces. The penetration of the cortical cells, however, is not general, but confined at first to a few strands of mycelium, which appear to be formed in the following manner. One hypha or a strand of a few hyphae pushes its way somewhat deeply into the tissue, travelling almost invariably through the middle lamellae. Other hyphae follow pushing their way alongside the original hypha which of course continues its growth. In this way a whole strand of mycelium is built up consisting of 20–30 or even more hyphae. Several such strands can frequently be found radiating out from an infected scab pustule. In Fig. 11 an excellent example of a young hyphal strand (*A*) penetrating from the subepidermal stroma (*C*) can be seen; in the same figure a more fully developed strand of many hyphae can be recognised at (*B*). In Fig 9 also it is possible to follow the subepidermal mycelium along from the place of the original scab infection towards the left, where a strand of mycelium is seen penetrating inwards towards the wood. These strands of mycelium are very characteristic of this type of *Nectria* infection. If the cork layer is not in an advanced stage of development, the *Nectria* hyphae are capable of penetrating between its cells through the middle lamellae of the cell walls. Fig. 12, which is a photograph of the section adjacent to that of Fig. 11 in the same series, shows such a stage. At (*D*) the mycelial strand consisting only of very few hyphae is penetrating the immature cork layer. The hyphae could be traced back to a much larger strand which is slightly out of focus in the photograph but which can be seen at (*B*) and which originated from the subepidermal mycelium (*C*). The young strand (*A*) (corresponding to (*A*) in Fig. 11) which has not yet reached the phellogen can just be distinguished. Once the mycelium has penetrated beyond the cork layer, the host sometimes makes a half-hearted attempt to stop its progress by the formation of a second phellogen as shown in Fig. 13. The *Nectria*, however, is powerful enough to penetrate the new phellogen in the same way as it did the old one, provided that cork formation has not yet taken place.

From the appearance of sections of old infections, the fungus seems to be capable of secreting some substance, probably of the nature of an enzyme, which is able to attack the cell walls of the cortical tissue. These are not totally destroyed but they lose their power of staining with Fuchsin and the tissue so affected appears indistinct and disorganised. Whether the secretion acts in advance of the *Nectria* mycelium is rather difficult to determine exactly but it appears to be probable, for cells on the outside of the infected area frequently have their contents coagulated and the cell walls rather heavily stained, before mycelium can be found among them. This stage, the first step in the disintegration of the tissue, is followed by the loss of the staining powers and by the advance of the mycelium in the middle lamellae between the cells in the intercellular spaces and in the cells themselves. The nature of the secretory substance has not been investigated. If present, it probably begins to take effect from the very early stages of infection and is perhaps a potent factor in overcoming the resistance of the host.

In this connection, it might be well to refer to some infection experiments carried out some years ago. In these it was sometimes found that if *Nectria* conidia were placed on superficial wounds on cut shoots under a bell-jar, the fungus penetrated to the cortex even when no intercellular spaces were exposed and before any cork layer was developed. The delay in forming a wound cork was considered to be due to the dormant and unhealthy condition of the host, as most of the experiments were made in winter and all of them on cut shoots which could not be expected to have the same vigour as the living tree. Normally *Nectria* mycelium grows in the intercellular spaces of the cortex and as these do not extend to the outside layers of the cortex except at lenticels it was obvious that in infecting through superficial cuts not over lenticels the fungus would have to pass through the cell tissue. The way this was brought about was by the solution of the middle lamellae of the cell walls. Repetition of the experiments during the summer, on growing trees, invariably resulted in a cork layer being formed round the infected portion and the latter completely excluded as mentioned above. It seems clear, therefore, that the secretions of the canker fungus cannot pass a well-formed cork layer, but if no such barrier exists or if it is only partially developed then the fungus can progress, apparently by the help of its secretions.

The later stages of infection. Once the canker fungus has effected an entrance to the cortex, it proceeds to grow very rapidly in all directions chiefly in the intercellular spaces. Concurrently with the gradual progress of the infection by the fungus the healthy cortical tissue usually becomes very active, its cells dividing rapidly and the intercellular spaces being more or less obliterated. This rapidly dividing tissue unless protected by a cork layer soon becomes infected with the canker mycelium, and undergoes changes described above. The host plant persists in its efforts to form a wound cork layer especially in the region between the sclerenchymatous bundles of the cortex, apparently to prevent the fungus from entering the wood. Sometimes the growth stimulus of the cells of the developing phellogen layer is so strong that the cells hypertrophy and the whole tissue becomes ruptured at this region. When the infection has penetrated too deeply to be excluded by a cork layer and cannot be prevented from reaching the wood, wound wood is formed, consisting of medullary ray-like cells, and these offer considerably more resistance to the path of the fungus than the vessels, since the contents of the brick-shaped cells of the wound wood become choked up with gummy material which especially collects at the pits through which the hyphae of *Nectria* normally pass. Sometimes the fungus reaches the wood before any wound wood can be formed and in this case wound wood is cut off from the cambium which still remains living round the infected area. In the cortex a strong cork layer is usually formed ultimately round the infected tissue and this has the effect of limiting the infection and is largely responsible for the concentric cracks in the canker scar which are so characteristic of the disease. Not infrequently the stem becomes completely girdled and the whole of the shoot above is killed off.

This manner of infection appears to be unusual amongst fungal parasites. Fungi parasitic on other fungi are known, but for one, unable to penetrate uninjured bark itself, to take advantage of the injury effected by another and subsequently supersede the latter is unique.

CONTROL.

The obvious way of controlling the infection of scab wounds by *Nectria* is to control the autumn infection of the scab fungus on the wood. It is the usual practice to spray against scab in the spring to protect the fruit and no measures beyond cutting out diseased wood are taken

Fig 1

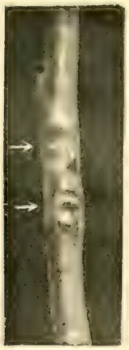


Fig. 2

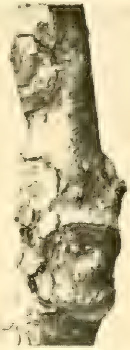


Fig. 3

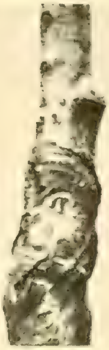


Fig. 4

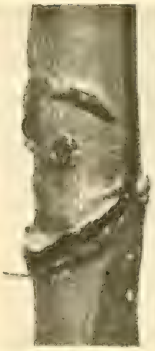


Fig. 5

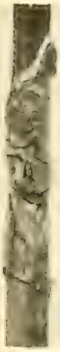


Fig. 6

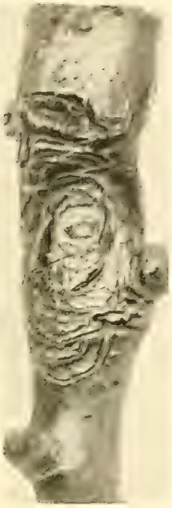


Fig. 7

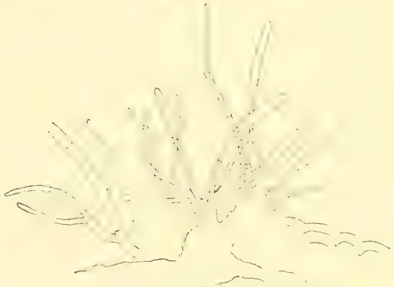


Fig. 8

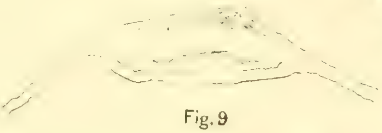


Fig. 9

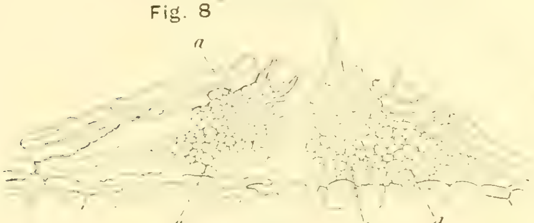


Fig. 10

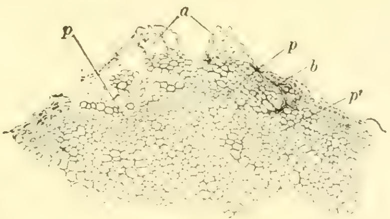


Fig. 13

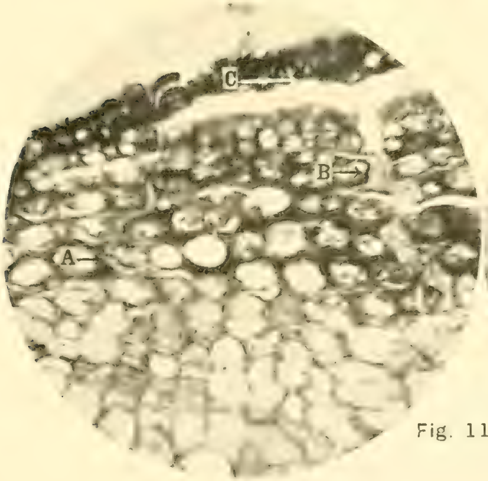


Fig. 11

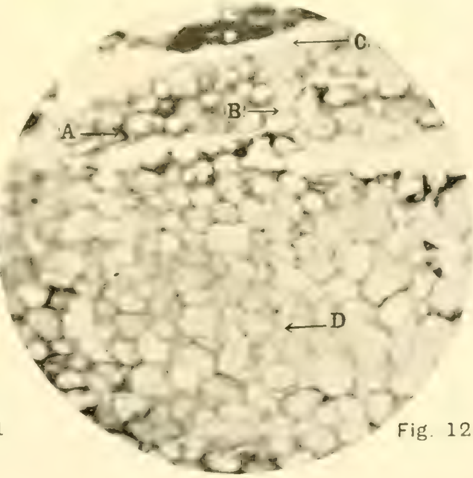


Fig. 12

against the autumn infection. Trials may show that winter spraying immediately after defoliation is effective.

Summary.

The infection of apple trees by the canker fungus through scab infections is described.

The conidia alighting on the exposed scab stroma give rise to a mycelium which attacks the latter and then grows out into the cortex. The fungus is able to pass through any immature cork layer and finally reach the wood.

EXPLANATION OF PLATE XII.

Fig. 1. Young stage in the canker infection of a scab wound. Variety, Lord Suffield. Jan. 16, 1922. × 1·5.

Fig. 2. Similar infections to Fig. 1, but slightly more advanced. Variety, Ecklinville. × 0·7.

Fig. 3. As Fig. 2, but further developed. Variety, Lord Suffield. Jan. 16, 1922. × 1·8.

Fig. 4. As Fig. 3. Variety, Lord Suffield. Jan. 16, 1922. Note the original scab from which the infection started. × 1·8.

Fig. 5. A very active infection on a vigorous shoot of Lord Suffield. Jan. 14, 1922. × 1·5.

Fig. 6. Very active canker in late stage of development. Variety, Lord Suffield. Jan. 16, 1922. × 1·5.

Fig. 7. Mature canker infection of scab wound. March 3, 1922. × 0·8.

Fig. 8. Camera lucida drawing of young canker pustule on outside of a scab infection. Variety, King of the Pippins. Nov. 1921. × 420.

Fig. 9. A trans. section through a young infection of a scab wound by *Nectria galligena*. King of the Pippins. Nov. 1921. × 38.

Fig. 10. Central portion of Fig. 9 enlarged to show the *Nectria* mycelium (*a, b*) growing on the *Venturia inaequalis* mycelium (*c, d*). Note the characteristic conidia of *Nectria galligena*. × 250.

Fig. 11. Trans. section of an infection showing the formation of hyphal strands of *Nectria galligena*. A young strand can be seen at *A*, and an older one at *B*, both strands being derived from the sub-epidermal mycelia *C*.

Fig. 12. Adjacent section to that of Fig. 11, showing the penetration of the young phellogen at *D*. The other lettering corresponds to that of Fig. 11.

Fig. 13. Trans. section through a canker infection of a scab pustule (*a*) showing the growth of the mycelial strand of *Nectria* (*b*) through the phellogen (*p*) into the cortex. A new phellogen (*p′*) is organised around the advancing mycelium. × 48.

REFERENCES.

Wiltshire, S. P. (1921). *Ann. App. Biol.* viii. 185.
—— (1913). *Brit. Ass. Report.*

(*Received April 6th*, 1922.)

THE INSECT AND OTHER INVERTEBRATE FAUNA OF ARABLE LAND AT ROTHAMSTED[1]

BY HUBERT M. MORRIS, M.Sc., F.E.S.

(*Entomological Dept., Institute of Plant Pathology, Rothamsted Experimental Station, Harpenden.*)

(With 7 Text-figures.)

CONTENTS.

		PAGE
1.	Description of the area examined	283
2.	Method of investigation	283
3.	Soil analyses	286
4.	Meteorological conditions	286
5.	Occurrence of weeds	287
6.	Soil fauna of the manured plot	288
7.	Census of manured plot	290
8.	Soil fauna of the control plot	293
9.	Census of control plot	295
10.	Comparison of the faunas of the two plots	296
11.	Distribution in depth	297
12.	Comparison with soil fauna of pasture land	300
13.	Relation of soil fauna to soil nitrogen	300
14.	The function of the invertebrate fauna in the soil	302
	Summary	303
	References	305

THIS investigation was carried out from February 1920, to January 1921, with the object of obtaining information as to the species of insects and other invertebrates present in the soil of an arable field. The various species and their relative numbers, the depth at which these organisms occur, and the effect upon them of the application of farmyard manure to the land were the principal points considered.

I am very much indebted to Dr A. D. Imms for suggestions and advice throughout this investigation. I am also indebted to Miss K. Warington for information regarding the weeds; to Mr G. C. Sawyer for estimating the nitrogen content of several groups; to Mr H. J. Page for

---

[1] A grant in aid of publication has been made for this communication.

the mechanical and chemical analyses; and for help in the identification of species of *Insecta*, *Myriapoda* and *Arachnida* to Messrs S. G. Brade-Birks, J. M. Brown, E. A. Butler, H. St J. K. Donisthorpe, F. W. Edwards, H. F. Fryer, J. E. Hull, R. C. L. Perkins.

1. DESCRIPTION OF THE AREA EXAMINED.

The area dealt with in this investigation was the Broadbalk field belonging to the Rothamsted Experimental Station, Harpenden. The soil of the Rothamsted fields is "clay with flints," which overlies chalk.

Broadbalk field is roughly rectangular in shape, the long sides running W.N.W. to E.S.E., and it lies on a gentle slope, the south-east side being the lowest, this side being slightly over 400 feet above sea level.

The field is divided into a number of plots, of which numbers 2 and 3 were dealt with in this investigation. Plot 2 has received annually a dressing of farmyard manure at the rate of fourteen tons to the acre since 1843. Plot 3, which is a control, has received no farmyard or artificial manure of any kind since the commencement of the experiments in 1843 and actually since 1839.

These plots are about half an acre in area, and lie side by side along the northern side of the field, being separated by a path two yards wide.

The effect of the different treatment of the plots is very noticeable in their yield of grain and straw, and in the general growth of the wheat and weeds. This treatment having been the same in either case for so many years makes them particularly well fitted for an investigation of the soil fauna which they support.

The plots were ploughed on October 13th, the manure having been applied to plot 2 just previously.

2. METHOD OF INVESTIGATION.

The samples of soil which were examined in the course of this investigation were taken from the western end of the plots, and were taken from the edges of the plots so as to disturb the soil of the plots as little as possible. Successive samples from the same plot were not taken next to each other, nor were any two samples taken nearer together than about a yard.

The method of taking the sample was as follows. Four iron plates were used, two of them twelve inches long by ten inches wide, one twelve inches long by nine inches wide, and one four inches long by nine inches wide. Each plate had an iron bar fastened to it at the top, and each of the three larger plates had two projecting teeth at the bottom. These teeth and the lower edges of the plates were kept sharpened in order that they might enter the ground more easily (Fig. 1).

The plates were driven into the ground to form a box nine inches square, the smallest plate being on the side towards the outside of the plot (Fig. 2). A hole was then dug in the path, extending about two feet from the smallest plate, and about a foot in width, in order to give room to remove the soil from the box. This hole was first made to a depth of about two inches, the front plate was then removed, and by means of the special trowel it was possible to remove the top layer of soil enclosed by the "box." This soil was then extracted to a depth of one inch;

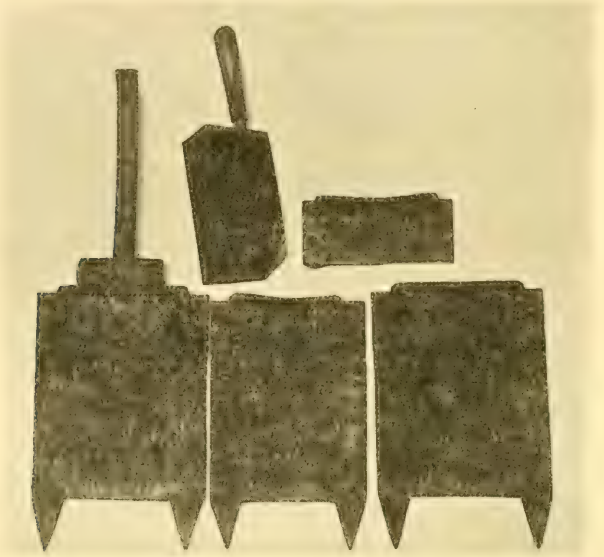


Fig. 1. Iron plates and trowel used in taking soil samples.

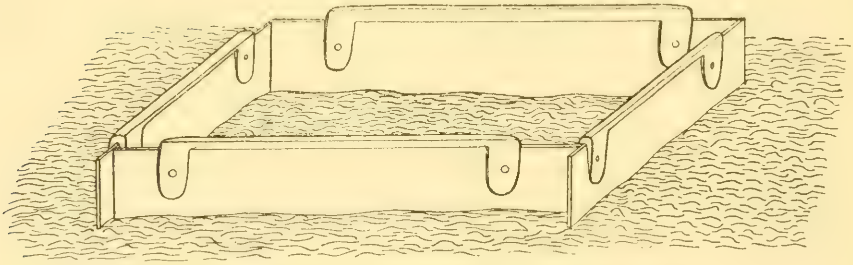


Fig. 2. Plates in position, before any soil has been removed.

owing to the unevenness of the soil the latter level was measured from the lowest point of the surface. On removal the soil was placed in a linen bag.

The small plate was then replaced and driven down another two inches, and the hole in the path was deepened by about another two inches (Fig. 3). The soil in the box was then removed in the same way as before. The second and succeeding samples were taken at depths of two inches at a time, each being placed in a separate bag.

The soil was removed in this way to a depth of nine inches, giving five samples, which consisted of—I, the soil between the surface and a depth of one inch below the lowest point of the surface; II, the soil between a depth of one inch below the lowest point of the surface and a depth of three inches; III, the soil between three inches and five inches; IV, the soil between five inches and seven inches; V, the soil between seven inches and nine inches.

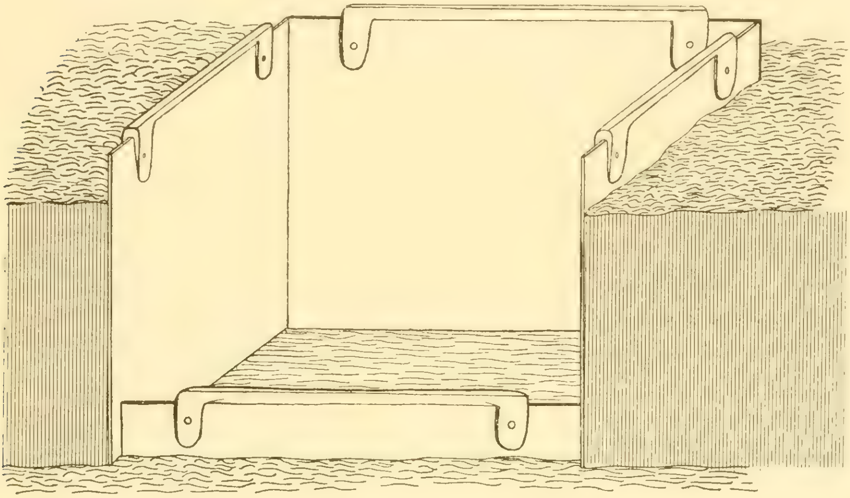


Fig. 3. Plates in position after three samples of soil have been removed.

The samples obtained in this way were taken to the laboratory for examination. When the soil was wet it was necessary to spread it out to dry for some time, before it was possible to examine it thoroughly. The examination had to be carried out by crumbling the soil on to sheets of brown paper, and watching for the appearance of insects, etc., as the soil was broken up. The soil was examined over brown paper instead of white, which at first might seem more suitable, because the most abundant small insects, and the majority of the larvae, were white or light-coloured. Other methods of obtaining the insects, etc., from the soil were considered, but were not found to be feasible. By taking a small quantity of soil at a time, and examining it in this way, it is possible to obtain, probably, practically all the insects, etc., from the soil, although it is likely that a few of the smaller forms would be overlooked.

Twenty-three cubes of soil, each 9″ × 9″ × 9″, were examined in this way, from each plot. They were taken alternately from the plots about every six days, so that a cube was taken from each plot about every 12 days.

The time between successive cubes, however, varied somewhat according to the weather and the condition of the soil. Cubes were not usually taken on rainy days owing to the difficulties entailed in the thorough examination of wet soil.

Since this investigation was completed a method has been devised by means of which the separation of insects and other arthropods from the soil is much facilitated (11).

3. Soil Analyses.

In order to define as exactly as possible the conditions under which the soil fauna was existing on the two plots examined, mechanical and chemical analyses of the soil of both plots were obtained.

Plot 2. Percentages. Moisture (in air-dry soil) 2·22; Nitrogen 0·258; Potash (soluble in HCl) 0·333; Phosphoric acid (soluble in HCl) 0·203; Lime (as $CaCO_3$) 3·43.

Fine gravel 1·63; Coarse sand 2·57; Fine sand 21·96; Silt 17·30; Fine silt I 11·66; Fine silt II 5·06; Clay 13·87; Loss on solution 7·38; Loss on ignition 11·95.

Plot 3. Percentages. Moisture (in air-dry soil) 1·7; Nitrogen 0·114; Potash (soluble in HCl) 0·284; Phosphoric acid (soluble in HCl) 0·099; Lime (as $CaCO_3$) 4·01.

Fine gravel 1·01; Coarse sand 3·17; Fine sand 23·31; Silt 20·36; Fine silt I 6·22; Fine silt II 3·81; Clay 16·56; Loss on solution 6·88; Loss on ignition 8·54.

The figure for loss on ignition includes combined moisture as well as organic matter.

4. Meteorological Conditions.

As the meteorological conditions probably exercise an influence on the soil fauna, especially the rainfall and soil temperature, records of these were obtained. The soil temperatures were registered by a recording thermometer at a depth of six inches.

These records have been preserved but are not considered in the present instance.

The total rainfall during the period February 1st, 1920, to January 29th, 1921, was 26·459 inches.

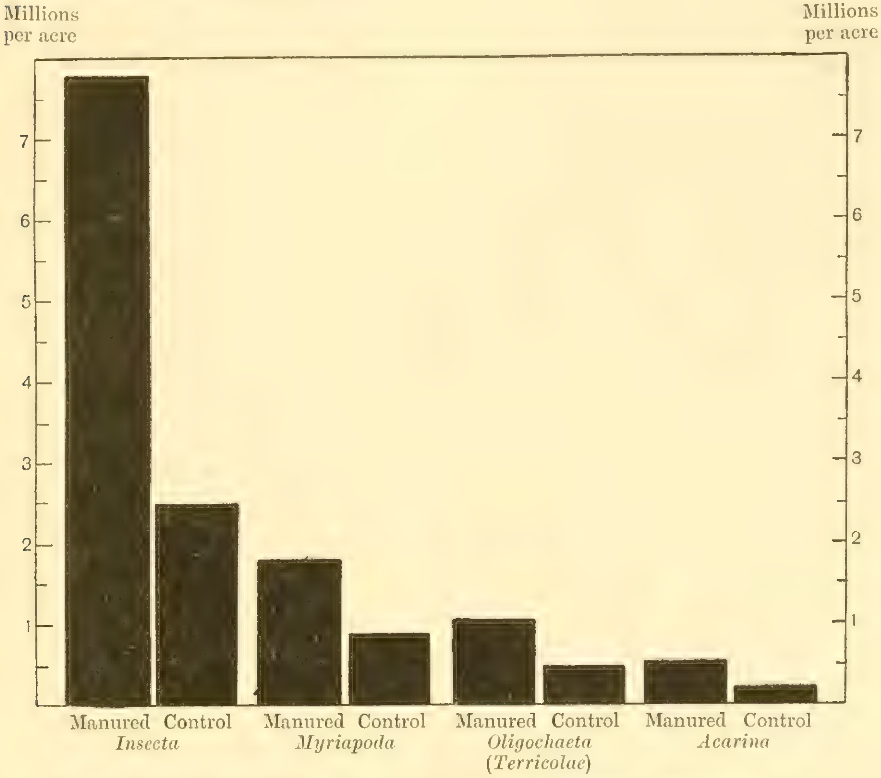


Fig. 4. Number of individuals in the more important groups in the manured and control plots.

5. OCCURRENCE OF WEEDS.

Plot 2. In the spring the most abundant weeds are *Veronica hederaefolia*, *Scandix pecten* and *Galium aparine*, and in addition to these *Alopecurus agrestis* and *Carduus arvensis* are plentiful.

In the summer *Scandix pecten* and *Galium aparine* are still abundant, and in addition to those occurring earlier, *Caucalis arvensis*, *Equisetum arvense* and *Tussilago farfara* are plentiful, and later still *Convolvulus arvensis* is also prevalent.

Plot 3. In the spring the most plentiful weeds are *Veronica hederaefolia* and *Galium aparine*. In the summer *Tussilago farfara*, *Sonchus arvensis*, *Vicia sativa* and *Lathyrus pratensis* are plentiful and *Alopecurus agrestis*, *Equisetum arvense*, *Carduus arvensis* and *Scabiosa arvensis* are generally distributed, and later still *Convolvulus arvensis* is also plentiful.

6. Soil Fauna of the Manured Plot.

In the following lists the worms have been divided into two groups, those belonging to the sub-order *Terricolae* of the order *Oligochaeta*, which includes the true earthworms, *Lumbricus*, etc., forming one group as *Oligochaeta* (*Terricolae*), and all other worms, probably principally belonging to the family *Enchytraeidae* of the *Oligochaeta*, and to the *Nematoda*, forming the second group as *Oligochaeta* (*Limicolae*), etc.

The numbers following the names have the following meaning—the first numbers give the months during which the species was met with. The first numbers within the brackets give, above, the total number found, and below, in Roman numerals, the levels in which they were found. The second numbers within the brackets give, above, the greatest number found at any one level, and below, in Roman numerals, the level at which they were found. Thus—*Trichocera fuscata* Mg. (larvae) 1–12 $\left(\frac{108}{\text{I–IV}}; \frac{54}{\text{II}}\right)$ indicates that larvae of *Trichocera fuscata* Mg. were found in each month from January to December; 108 were found altogether in samples I, II, III and IV, *i.e.* between the surface and a depth of seven inches, and that 54 of these were found in the second sample, between a depth of one inch and three inches.

The species of insects and other invertebrates present in the manured plot are as follows:

INSECTA.

Collembola. Onychiuridae. *Onychiurus fimetarius* (Linn.) 1–12; *O. ambulans* (Linn.) 1–12; *Tullbergia quadrispina* (Börn.) 2, 9.

Isotomidae. *Isotoma viridis* Bourl. 2–3, 8–10; *I. minor* Schäff. 1, 4, 5, 10; *I. olivacea* (Tullb.) 2; *Folsomia quadrioculata* (Tullb.) 4; *Isotomurus palustris* (Müll.) 10.

Entomobryidae. *Entomobrya multifasciata* (Tullb.) 8; *Lepidocyrtus cyaneus* (Tullb.) 1, 5, 8; *L. albus* Pack. 4; *Orchesella villosa* (Geoff.) 8, 10; *Heteromurus nitidus* Templ. 2, 4, 8, 10, 12.

Smynthuridae. *Smynthurus viridis* (Linn.) 8.

Collembola. All species 1–12 $\left(\frac{710}{\text{I–V}}; \frac{264}{\text{II}}\right)$.

Thysanura. Campodeidae. *Campodea staphylinus* Westw.; *C. gardneri* Bagn.; *C. fragilis* Meinert. Spp. 2–11 $\left(\frac{33}{\text{I–IV}}; \frac{14}{\text{II}}\right)$.

Orthoptera. Forficulidae. *Forficula auricularia* L. 2, 6, 8, 10 $\left(\frac{4}{\text{I–II}}\right)$.

Thysanoptera. Spp. 5–8 $\left(\frac{7}{\text{I}}\right)$.

Hemiptera. Capsidae. *Lygus pastinacae* Fall. 4 $\left(\frac{1}{\text{I}}\right)$.

Aphididae. *Aphis* sp. 9 $\left(\frac{1}{\text{I}}\right)$.

Lepidoptera. Hepialidae. Unidentified larvae 1–5, 8–12 $\left(\frac{7}{\text{I–V}}; \frac{3}{\text{II}}\right)$.

Unidentified larvae 3, 8 $\left(\frac{2}{\text{I}}\right)$.

Coleoptera. Carabidae. *Notiophilus aquaticus* L. 4 $\left(\frac{1}{\text{I}}\right)$; *Badister bipustulatus* F. 3 $\left(\frac{1}{\text{I}}\right)$; *Bradycellus verbasci* Duft. 8 $\left(\frac{2}{\text{I}}\right)$; *Harpalus ruficornis* F. 5 $\left(\frac{1}{\text{I}}\right)$; *H. aenus* F. 2 $\left(\frac{1}{\text{I}}\right)$; *Pterostichus madidus* F. 4–6 $\left(\frac{5}{\text{I–III}}; \frac{3}{\text{II}}\right)$; *Bembidium guttula* F. 2 $\left(\frac{1}{\text{I}}\right)$.

Hydrophylidae. *Helophorus nubilus* F. 5, 6, 8, 9 $\left(\frac{5}{\text{I}}\right)$.

Staphylinidae. *Homalota* spp. 2, 3, 5, 9, 11 $\left(\frac{7}{\text{I–V}}\right)$; *Tachyporus hypnorum* F. 5, 8 $\left(\frac{4}{\text{I}}\right)$; *Quedius cinctus* Payk. 4, 5 $\left(\frac{2}{\text{I, II}}\right)$; *Ocypus morio* Grav. 9 $\left(\frac{1}{\text{II}}\right)$; *Philonthus trossulus* Nord. 9 $\left(\frac{1}{\text{I}}\right)$; *Lathrobium fulvipenne* Grav. 2 $\left(\frac{1}{\text{IV}}\right)$; *L. longulum* Grav. 10 $\left(\frac{1}{\text{V}}\right)$; *Scopaeus* sp. 8 $\left(\frac{1}{\text{II}}\right)$; *Medon propinquus* Bris. 5 $\left(\frac{1}{\text{I}}\right)$; *Stenus subaeneus* Er. 10 $\left(\frac{1}{\text{I}}\right)$; *Oxytellus laqueatus* Marsh. 6, 11 $\left(\frac{6}{\text{I–IV}}; \frac{2}{\text{I, IV}}\right)$; *O. inustus* Grav. 9 $\left(\frac{1}{\text{I}}\right)$; *O. sculpturatus* Grav. 4, 6, 9 $\left(\frac{5}{\text{I–IV}}; \frac{2}{\text{III}}\right)$; *O. nitidulus* Grav. 10 $\left(\frac{1}{\text{III}}\right)$; *O. tetracarinatus* Block. 3 $\left(\frac{1}{\text{III}}\right)$.

Pselaphidae. *Bryaxis fossulata* Reich. 3 $\left(\frac{1}{\text{I}}\right)$.

Lathridiidae. *Enicmus minutus* L. 10 $\left(\frac{1}{\text{III}}\right)$; *Melanophthalma fuscula* Humm. 6 $\left(\frac{1}{\text{I}}\right)$.

Cucujidae. *Silvanus surinamensis* L. 6 $\left(\frac{1}{\text{I}}\right)$.

Elateridae. *Agriotes sputator* L. 3, 5 $\left(\frac{2}{\text{I}}\right)$.

Chrysomelidae. *Phyllotreta undulata* Kuts. 8 $\left(\frac{1}{\text{I}}\right)$; *Plectroscelis concinna* Marsh. 6 $\left(\frac{1}{\text{I}}\right)$.

Curculionidae. *Sitones humeralis* Steph. 8 $\left(\frac{1}{\text{I}}\right)$.

Larvae and Pupae—Carabidae 1, 4, 5, 9–12 $\left(\frac{14}{\text{I–V}}; \frac{9}{\text{II}}\right)$; Staphylinidae 1–6, 10–12 $\left(\frac{103}{\text{I–V}}; \frac{36}{\text{I}}\right)$; Elateridae 1–12 $\left(\frac{59}{\text{I–V}}; \frac{19}{\text{IV}}\right)$; Telephoridae 1, 3, 8, 10 $\left(\frac{4}{\text{I, V}}\right)$; Curculionidae 10, 11 $\left(\frac{2}{\text{III, IV}}\right)$; unidentified 4, 12 $\left(\frac{4}{\text{I, II}}\right)$.

Diptera. Mycetophilidae. *Sciara* sp. 12 $\left(\frac{1}{\text{III}}\right)$.

Chironomidae. *Camptocladius aterrimus* Mg. (Reared from larvae.)

Tipulidae. *Pachyrrhina maculosa* Mg. (larva) 4 $\left(\frac{1}{\text{I}}\right)$; *P. histrio* F. (larva) 5 $\left(\frac{1}{\text{I}}\right)$; *Trichocera fuscata* Mg. (larvae) 1–12 $\left(\frac{108}{\text{I–IV}}; \frac{54}{\text{II}}\right)$.

Scatopsidae. *Scatopse halterata* Mg. (larvae) 1 $\left(\frac{2}{\text{III, IV}}\right)$.

Empidae. Sp. reared from larvae.

Unidentified larvae of the following families also occurred:

Cecidomyidae 1–12 $\left(\frac{58}{\text{I–IV}}; \frac{39}{\text{I}}\right)$; Mycetophilidae 1–12 $\left(\frac{35}{\text{I–IV}}; \frac{12}{\text{III}}\right)$; Chironomidae 1–6, 9–12 $\left(\frac{153}{\text{I–III}}; \frac{92}{\text{II}}\right)$; Tipulidae 4, 5, 10, 11 $\left(\frac{5}{\text{I–III}}; \frac{4}{\text{I}}\right)$; Empidae 1–12 $\left(\frac{48}{\text{I–IV}}; \frac{25}{\text{II}}\right)$; Syrphidae 1, 4, 9 $\left(\frac{3}{\text{I}}\right)$; Anthomyidae 3, 9–12 $\left(\frac{9}{\text{I–III}}; \frac{4}{\text{II, III}}\right)$.

Hymenoptera. Chalcididae. One species, unidentified 6 $\left(\frac{1}{\text{III}}\right)$.

FORMICIDAE. *Myrmecina graminicola* Fabr. 2, 8 $\left(\frac{32}{\text{I–V}};\ \frac{17}{\text{IV}}\right)$; *Myrmica laevinodis* Nyl. 4–6, 9–10 $\left(\frac{835}{\text{I–V}};\ \frac{759}{\text{I}}\right)$; *Acanthomyops* (*Donisthorpea*) *nigra* L. 8 $\left(\frac{8}{\text{I–II}}\right)$.

ANDRENIDAE. *Andrena chrysosceles* Kirby 3 $\left(\frac{1}{\text{III}}\right)$.

"MYRIAPODA[1]."

DIPLOPODA. *Brachydesmus superus mosellanus* Verhoeff 1–12 $\left(\frac{96}{\text{I–V}};\ \frac{30}{\text{III}}\right)$; *Cylindroiulus londinensis* var. *caeruleocinctus* (Wood) (=*C. londinensis* var. *teutonicus* (Pocock) of some records) 1–12 $\left(\frac{129}{\text{I–V}};\ \frac{41}{\text{II}}\right)$; *Blaniulus guttulatus* (Bosc.) 1–12 $\left(\frac{138}{\text{I–V}};\ \frac{34}{\text{V}}\right)$; *Archiboreoiulus pallidus* Brade-Birks 2, 3, 6–12 $\left(\frac{40}{\text{I–V}};\ \frac{15}{\text{II}}\right)$.

CHILOPODA. *Lithobius* sp. 8 $\left(\frac{1}{\text{I}}\right)$; *Geophilus longicornis* Leach 1–12 $\left(\frac{57}{\text{I–V}};\ \frac{23}{\text{II}}\right)$.

SYMPHYLA. Spp. 1–12 $\left(\frac{64}{\text{I–V}};\ \frac{32}{\text{V}}\right)$.

ARACHNIDA.

Areinida. *Porrhomma pygmaeum* Pd. 4 $\left(\frac{1}{\text{I}}\right)$; *P. microphthalmum* Cb. 10 $\left(\frac{1}{\text{IV}}\right)$; *Robertus lividus* Bl. 6 $\left(\frac{1}{\text{III}}\right)$; *Linyphia* spp. 6, 8 $\left(\frac{2}{\text{I, III}}\right)$; *Oedothorax agrestris* Bl. 3 $\left(\frac{1}{\text{I}}\right)$.

Acarina. ANYSTIDAE. *Anystis baccarum* L. 5, 6 $\left(\frac{3}{\text{I}}\right)$.

GAMASIDAE. *Gamasus magnus* Kr. 4–6, 9–12 $\left(\frac{10}{\text{I–III}};\ \frac{6}{\text{I}}\right)$; *Gamasus* sp. (immature) 2–5, 8–9 $\left(\frac{13}{\text{I–III}};\ \frac{7}{\text{I}}\right)$; *Pergamasus crassipes* L. 4–12 $\left(\frac{11}{\text{I–IV}};\ \frac{7}{\text{I}}\right)$; *P. meridionalis* Berl. 10 $\left(\frac{1}{\text{I}}\right)$; *P. hamatus* Koch 3–5, 8–11 $\left(\frac{14}{\text{I–V}};\ \frac{5}{\text{II}}\right)$; *P. septrionalis* Oud. 1, 5–12 $\left(\frac{33}{\text{I–III}};\ \frac{19}{\text{I}}\right)$; *P. rumiger* Berl. 5, 8, 9 $\left(\frac{5}{\text{I–II}};\ \frac{4}{\text{I}}\right)$; *P. alpestris* Berl. 10 $\left(\frac{1}{\text{I}}\right)$; *Pergamasus* spp. (immature) 1–5, 8–10 $\left(\frac{40}{\text{I–IV}};\ \frac{17}{\text{I}}\right)$; *Pachylaelaps pectinifer* Berl. 4 $\left(\frac{1}{\text{II}}\right)$.

TARSONEMIDAE. *Pigmephorus morrisii* Hull. 2, 8 $\left(\frac{4}{\text{II, III}};\ \frac{3}{\text{III}}\right)$.

TYROGLYPHIDAE. *Rhizoglyphus echinopus* Rob. 3 $\left(\frac{5}{\text{II}}\right)$; *Histiostoma julorum* Koch (hypopus) 3 $\left(\frac{1}{\text{II}}\right)$.

OLIGOCHAETA (*Terricolae*) 1–12 $\left(\frac{300}{\text{I–V}};\ \frac{111}{\text{II}}\right)$.

OLIGOCHAETA (*Limicolae*), **NEMATODA**, etc. 1–12 $\left(\frac{1072}{\text{I–V}};\ \frac{403}{\text{II}}\right)$.

ISOPODA 4, 5, 8–11 $\left(\frac{24}{\text{I–V}};\ \frac{7}{\text{II}}\right)$.

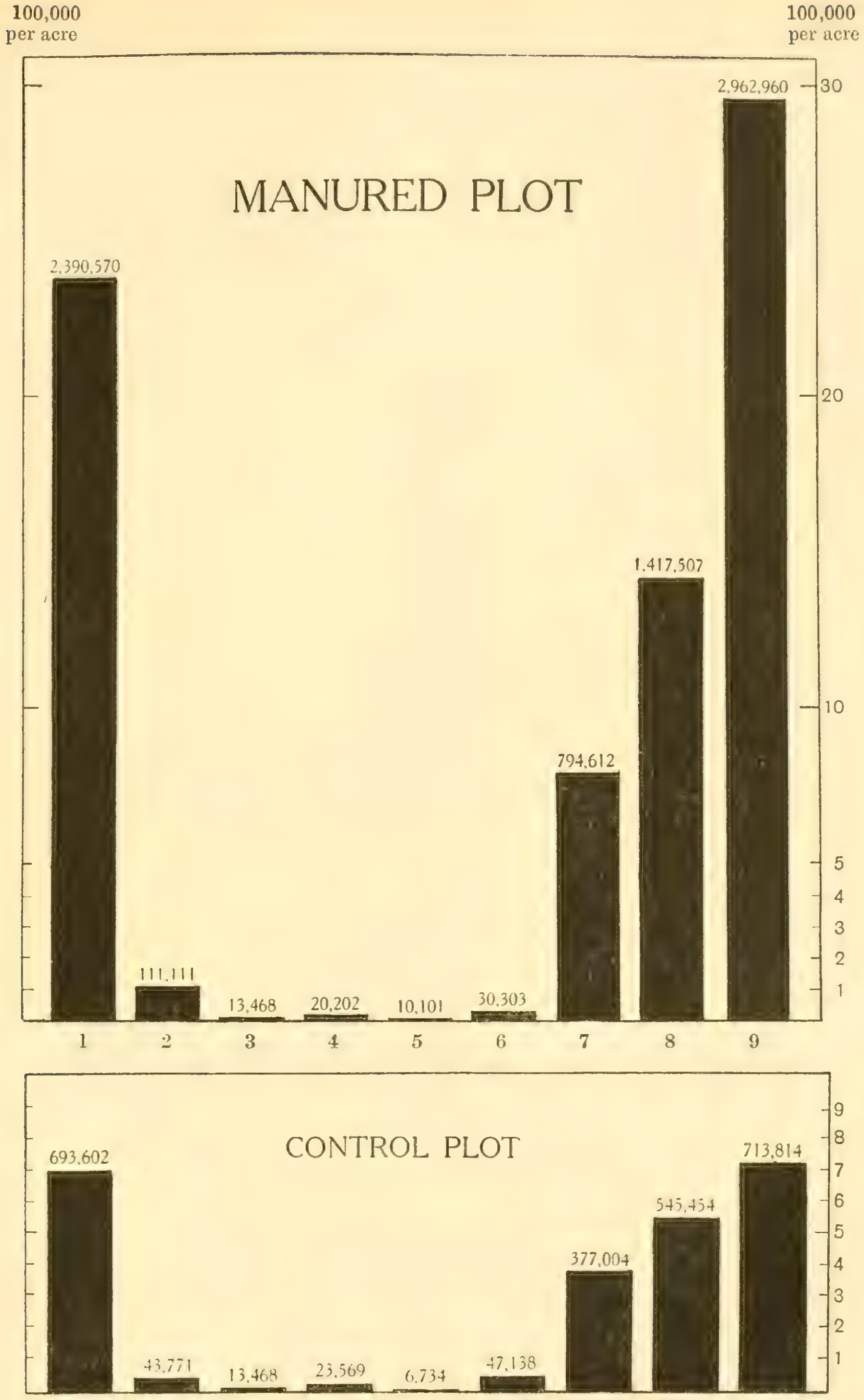
GASTROPODA 2, 4 $\left(\frac{10}{\text{I, III}};\ \frac{6}{\text{II}}\right)$.

7. CENSUS OF MANURED PLOT.

The total number of invertebrates found in plot 2, in twenty-three samples, was 4485, or 15,100,955 per acre. Of these 2295 were insects, or 7,727,265 per acre.

---

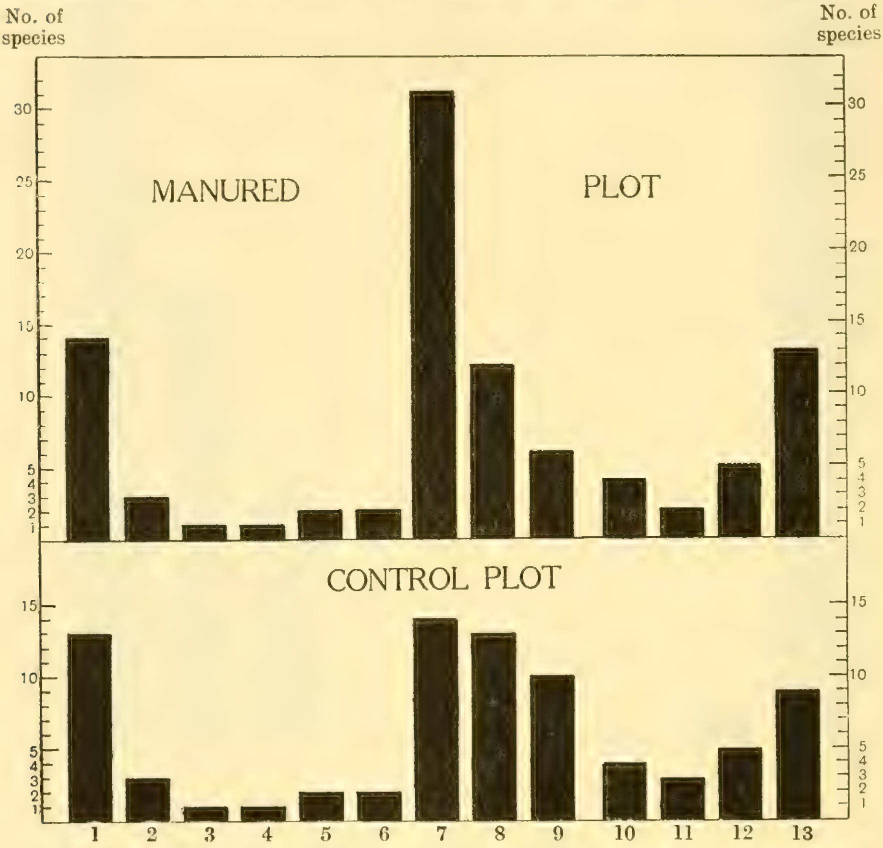
[1] The old term "Myriapoda" is used for convenience to include the classes *Diplopoda*, *Chilopoda* and *Symphyla*.



1, *Collembola*; 2, *Thysanura*; 3, *Orthoptera*; 4, *Thysanoptera*; 5, *Hemiptera*; 6, *Lepidoptera*; 7, *Coleoptera*; 8, *Diptera*; 9, *Hymenoptera*.

Fig. 5. Number of individuals in the different orders of insects in the manured and control plots.

The numbers per acre of the more abundant groups were as follows: *Oligochaeta* (*Limicolae*), etc., 3,609,424; *Formicidae* 2,946,125; *Collembola* 2,390,570; *Diplopoda* 1,367,002; *Oligochaeta* (*Terricolae*) 1,010,101; *Acarina* 531,986; *Chironomidae* (larvae) 515,151. The numbers of insects belonging to groups which are recognised as pests were: *Elateridae* (larvae) 198,653; *Tipulidae* (larvae) 16,835; *Hepialidae* (larvae) 23,569. The numbers per acre in the different orders are shown in Fig. 5. The "probable error" in the total population per acre is ± 1,700,000, and in the number of *Elateridae* larvae per acre ± 22,000.



1, *Collembola*; 2, *Thysanura*; 3, *Orthoptera*; 4, *Thysanoptera*; 5, *Hemiptera*; 6, *Lepidoptera*; 7, *Coleoptera*; 8, *Diptera*; 9, *Hymenoptera*; 10, *Diplopoda*; 11, *Chilopoda*; 12, *Areinida*; 13, *Acarina*.

Fig. 6. Number of species in the different orders in the manured and control plots.

The number of species of insects which occurred in the samples was about 72 but the number may have been slightly higher, as all the larvae found could not be exactly determined. The average number of insects per sample was 99·78.

The following orders occurred in the percentages given: *Collembola* 30·84; *Thysanura* 1·43; *Orthoptera* 0·17; *Thysanoptera* 0·26; *Hemiptera* 0·13; *Lepidoptera* 0·39; *Coleoptera* 10·25; *Diptera* 18·29; *Hymenoptera* 38·22.

The dominant order in respect of number of species present was the *Coleoptera*, with 31 species. The numbers of species in the different orders are shown in Fig. 6.

The most abundant species were *Myrmica laevinodis*, which made up 36·3 per cent. of the total insects; *Onychiurus ambulans*, 13·9 per cent. of the total; and *Onychiurus fimetarius*, 13·2 per cent. of the total.

Six species of *Myriapoda* (excluding *Symphyla*) occurred in this plot, and seventeen species of *Arachnida*.

8. Soil Fauna of the Control Plot.

The species of insects and other invertebrates present in the control plot are as follows:

INSECTA.

Collembola. ONYCHIURIDAE. *Onychiurus fimetarius* (Linn.) 1–10; *O. ambulans* (Linn.) 1–11; *Tullbergia quadrispina* (Börn.) 3, 4, 6, 10, 11.

ISOTOMIDAE. *Isotoma viridis* Bourl. 6, 9, 10; *I. minor* Schäff. 3, 4; *Folsomia quadrioculata* (Tullb.) 4; *Isotomurus palustris* (Müll.) 4, 10; *I. palustris* var. *aquatilis* 9.

ENTOMOBRYIDAE. *Entomobrya multifasciata* (Tullb.) 9; *Lepidocyrtus cyaneus* (Tullb.) 1, 10, 11; *L. albus* Pack. 3, 4, 9; *Orchesella villosa* (Geoff.) 1, 6, 9, 10, 12; *Heteromurus nitidus* Templ. 3, 4, 9, 10.

Collembola. All species 1–12 $\left(\frac{206}{\text{I–V}}; \frac{92}{\text{II}}\right)$.

Thysanura. CAMPODEIDAE. *Campodea staphylinus* Westw.; *C. gardneri* Bagn.; *C. fragilis* Meinert. Spp. 3–11 $\left(\frac{13}{\text{I–IV}}; \frac{6}{\text{II}}\right)$.

Orthoptera. FORFICULIDAE. *Forficula auricularia* L. 2, 6 $\left(\frac{4}{\text{I–IV}}; \frac{2}{\text{II}}\right)$.

Thysanoptera. Spp. 6, 11 $\left(\frac{6}{\text{I}}\right)$.

Hemiptera. JASSIDAE. *Cicadula sexnotata* Fall. 9 $\left(\frac{1}{\text{I}}\right)$.

CIMICIDAE. *Lyctocoris campestris* Fall. 6 $\left(\frac{1}{\text{I}}\right)$.

Lepidoptera. HEPIALIDAE. Unidentified larvae 2–4, 9 $\left(\frac{7}{\text{I–V}}; \frac{2}{\text{II, III, V}}\right)$.

TINEIDAE. Unidentified larva 8 $\left(\frac{1}{\text{II}}\right)$.

Unidentified larvae 3, 4, 9 $\left(\frac{6}{\text{I, II}}; \frac{5}{\text{I}}\right)$.

Coleoptera. CARABIDAE. *Clivina fossor* L. 4 $\left(\frac{1}{\text{II}}\right)$; *Bembidium guttula* F. 9 $\left(\frac{1}{\text{I}}\right)$.

HYDROPHYLIDAE. *Helophorus nubilus* F. 5 $\left(\frac{1}{\text{I}}\right)$.

STAPHYLINIDAE. *Homalota* spp. 2, 3, 6, 9, 10 $\left(\frac{6}{\text{I–IV}}; \frac{2}{\text{I, II}}\right)$; *Tachyporus hypnorum* F. 8

$\left(\frac{1}{\text{II}}\right)$; *Philonthus agilis* Grav. 9 $\left(\frac{1}{\text{I}}\right)$; *P. trossulus* Nord. 10 $\left(\frac{1}{\text{I}}\right)$; *Lathrobium longulum* Grav. 6, 9, 10 $\left(\frac{5}{\text{II--V}}; \frac{3}{\text{III}}\right)$; *Medon propinquus* Bris. 4 $\left(\frac{1}{\text{I}}\right)$; *Stenus subaeneus* Er. 5, 9 $\left(\frac{3}{\text{I}}\right)$; *Oxytellus insecatus* Grav. 2, 3 $\left(\frac{2}{\text{I--III}}\right)$.

CUCUJIDAE. *Silvanus surinamensis* L. 10 $\left(\frac{1}{\text{I}}\right)$.

ELATERIDAE. *Agriotes sputator* L. 3, 8 $\left(\frac{3}{\text{I--III}}\right)$.

CURCULIONIDAE. *Sitones humeralis* Steph. 2, 3, 8–10 $\left(\frac{10}{\text{I}}\right)$.

LARVAE AND PUPAE—CARABIDAE 4 $\left(\frac{1}{\text{II}}\right)$; STAPHYLINIDAE 2–4, 9–11 $\left(\frac{16}{\text{I--IV}}; \frac{8}{\text{I}}\right)$; SCARABAEIDAE 10 $\left(\frac{1}{\text{I}}\right)$; ELATERIDAE 1–12 $\left(\frac{49}{\text{I--V}}; \frac{16}{\text{IV}}\right)$; TELEPHORIDAE 10 $\left(\frac{1}{\text{III}}\right)$; CURCULIONIDAE, *Sitones humeralis* Steph. 9 $\left(\frac{1}{\text{I}}\right)$; Unidentified 4 $\left(\frac{1}{\text{II}}\right)$.

Diptera. CECIDOMYIDAE. *Campylomyza* sp. (larva) 2 $\left(\frac{1}{\text{I}}\right)$.

MYCETOPHILIDAE. *Sciara* sp. 9 $\left(\frac{1}{\text{I}}\right)$.

CHIRONOMIDAE. *Camptocladius aterrimus* Mg. Reared from larvae.

TIPULIDAE. *Trichocera fuscata* Mg. (larvae) 6–9 $\left(\frac{6}{\text{I--III}}; \frac{4}{\text{I}}\right)$.

BIBIONIDAE. *Dilophus febrilis* L. 9 $\left(\frac{1}{\text{I}}\right)$.

CHLOROPIDAE. Sp. 9 $\left(\frac{1}{\text{I}}\right)$.

Unidentified larvae of the following families also occurred:

CECIDOMYIDAE 1–6, 9–10 $\left(\frac{63}{\text{I--V}}; \frac{28}{\text{I}}\right)$; MYCETOPHILIDAE 2, 3, 9–11 $\left(\frac{23}{\text{I--V}}; \frac{10}{\text{V}}\right)$; CHIRONOMIDAE 3–5, 9, 10 $\left(\frac{8}{\text{I--IV}}; \frac{5}{\text{II}}\right)$; TIPULIDAE 1, 10, 12 $\left(\frac{5}{\text{I, II}}; \frac{4}{\text{II}}\right)$; SCATOPSIDAE 3 $\left(\frac{7}{\text{II, IV}}; \frac{6}{\text{IV}}\right)$; EMPIDAE 1–4, 9, 11 $\left(\frac{15}{\text{I--IV}}; \frac{8}{\text{II}}\right)$; SYRPHIDAE 3, 10 $\left(\frac{4}{\text{I, II}}; \frac{3}{\text{I}}\right)$; ANTHOMYIDAE 2, 10 $\left(\frac{7}{\text{I}}\right)$.

Hymenoptera. TENTHREDINIDAE. Unidentified larvae of two species 1, 6, 9 $\left(\frac{3}{\text{I--V}}; \frac{2}{\text{I}}\right)$.

CHALCIDIDAE. Three species, unidentified, 6, 10 $\left(\frac{3}{\text{I--III}}; \frac{2}{\text{I}}\right)$.

ICHNEUMONIDAE. *Pezomachus costatus* Bridge 9 $\left(\frac{1}{\text{I}}\right)$.

FORMICIDAE. *Myrmecina graminicola* Fabr. 10 $\left(\frac{1}{\text{I}}\right)$; *Myrmica laevinodis* Nyl. 3, 4, 6, 9 $\left(\frac{205}{\text{I--V}}; \frac{159}{\text{III}}\right)$; *Acanthomyops* (*Donisthorpea*) *nigra* L. 6 $\left(\frac{1}{\text{I}}\right)$.

ANDRENIDAE. *Andrena chrysosceles* Kirby 11 $\left(\frac{3}{\text{III, IV}}; \frac{2}{\text{III}}\right)$.

"MYRIAPODA."

DIPLOPODA. *Brachydesmus superus mosellanus* Verhoeff 1–5, 8–11 $\left(\frac{65}{\text{I--IV}}; \frac{26}{\text{I}}\right)$; *Cylindroiulus londinensis* var. *caeruleocinctus* (Wood) (= *C. londinensis* var. *teutonicus* (Pocock) of some records) 2–6, 9, 10 $\left(\frac{53}{\text{I--V}}; \frac{20}{\text{I}}\right)$; *Blaniulus guttulatus* (Bosc.) 1, 4–11 $\left(\frac{34}{\text{I--V}}; \frac{13}{\text{V}}\right)$; *Archiboreoiulus pallidus* Brade-Birks 1–12 $\left(\frac{25}{\text{I--V}}; \frac{7}{\text{V}}\right)$.

CHILOPODA. *Lithobius* sp. 6 $\left(\frac{1}{\text{I}}\right)$; *Geophilus longicornis* Leach 1–12 $\left(\frac{67}{\text{I--V}}; \frac{21}{\text{IV}}\right)$; *Geophilomorph* 2 $\left(\frac{1}{\text{III}}\right)$.

SYMPHYLA. Spp. 2–4, 9–11 $\left(\frac{19}{\text{II--V}}; \frac{6}{\text{IV, V}}\right)$.

ARACHNIDA.

Areinida. *Porrhomma pygmaeum* Pd. 3 $\binom{1}{\text{III}}$; *Centromerus bicolor* Bl. 3 $\binom{1}{\text{II}}$; *Trochosa terricola* Thor. 8, 9 $\binom{2}{\text{I}}$; *Stemonyphantes lineatus* L. 10 $\binom{1}{\text{III}}$; *Linyphia* spp. 9 $\binom{1}{\text{I}}$.

Acarina. ANYSTIDAE. *Anystis baccarum* L. 6 $\left(\frac{1}{\text{I}}\right)$.

GAMASIDAE. *Gamasus magnus* Kr. 1, 3–6, 9–12 $\left(\frac{14}{\text{I–III}}; \frac{9}{\text{I}}\right)$; *Gamasus* sp. (immature) 3, 4 $\left(\frac{2}{\text{I}}\right)$; *Pergamasus crassipes* L. 6, 10 $\left(\frac{7}{\text{I–III}}; \frac{6}{\text{I}}\right)$; *P. crassipes* var. *longicornis* 2, 10 $\left(\frac{4}{\text{I, II}}; \frac{3}{\text{I}}\right)$; *P. meridionalis* Berl. 11 $\left(\frac{1}{\text{II}}\right)$; *P. hamatus* Koch 6 $\left(\frac{1}{\text{III}}\right)$; *P. septrionalis* Oud. 6, 9, 12 $\left(\frac{6}{\text{I}}\right)$; *P. rumiger* Berl. 5, 10 $\left(\frac{2}{\text{I}}\right)$; *Pergamasus* spp. (immature) 3–11 $\left(\frac{13}{\text{I–IV}}; \frac{4}{\text{I, II}}\right)$.

OLIGOCHAETA (*Terricolae*) 1–12 $\left(\frac{136}{\text{I–V}}; \frac{56}{\text{II}}\right)$.

OLIGOCHAETA (*Limicolae*), **NEMATODA**, etc. 1–12 $\left(\frac{236}{\text{I–V}}; \frac{75}{\text{III}}\right)$.

ISOPODA 1, 3, 9–11 $\left(\frac{10}{\text{I–V}}; \frac{3}{\text{V}}\right)$.

GASTROPODA 1, 4, 10, 11 $\left(\frac{4}{\text{I–III}}; \frac{3}{\text{I}}\right)$.

Silvanus surinamensis L., which is recorded in the foregoing lists as having occurred once in the soil from each plot, is an introduced species which is usually recorded as having been found in stored foodstuffs, although Fowler (4) states that it has been taken under the bark of trees in Yorkshire, Epping Forest and Scotland. It seems doubtful if the specimens met with in the present instance could have been living in the soil; they may possibly have entered the soil in the laboratory before it was examined.

9. CENSUS OF CONTROL PLOT.

The total number of invertebrates found in plot 3, in twenty-three samples, was 1471 or 4,952,857 per acre. Of these 735, or 2,474,745 per acre, were insects.

The numbers per acre of the more abundant groups were as follows: *Oligochaeta* (*Limicolae*), etc., 794,612; *Collembola* 693,602; *Formicidae* 690,235; *Diplopoda* 595,959; *Oligochaeta* (*Terricolae*) 457,912; *Acarina* 215,488; *Chilopoda* 215,488. The numbers of insects belonging to groups recognised as pests were: *Elateridae* (larvae) 164,983; *Hepialidae* (larvae) 23,569; and *Tipulidae* (larvae) 16,835. The numbers per acre in the different orders are shown in Fig. 5.

The "probable error" in the total population per acre is ± 520,000, and in the number of *Elateridae* larvae per acre ± 44,000.

The number of species of insects which occurred in the samples was about 60 but, as in the other plot, this number might have been higher if all the larvae could have been exactly determined.

The average number of insects per sample was 31·95.

The following orders were represented in the percentages given: *Collembola* 28·14; *Thysanura* 1·78; *Orthoptera* 0·55; *Thysanoptera* 0·96; *Hemiptera* 0·27; *Lepidoptera* 1·91; *Coleoptera* 15·30; *Diptera* 22·13; *Hymenoptera* 28·96.

The dominant order in number of species present was the *Coleoptera*, with 14 species. The number of species in the different orders is shown in Fig. 6.

The most abundant species of insects were *Myrmica laevinodis*, which made up 27·9 per cent. of the total insects, *Onychiurus ambulans* 6·8 per cent., and *Onychiurus fimetarius* 6·5 per cent.

Seven species of *Myriapoda* (excluding *Symphyla*) occurred in this plot, and thirteen species of *Arachnida*.

10. COMPARISON OF THE FAUNAS OF THE TWO PLOTS.

It is noticeable that in both the plots the *Oligochaeta* (*Limicolae*), *Formicidae* and *Collembola* were much the most abundantly represented groups, and that the *Diplopoda*, *Oligochaeta* (*Terricolae*) and *Acarina* were also very numerous in both plots. There was not very much difference between the numbers of *Elateridae* larvae in the two plots, the numbers being 198,653 per acre in plot 2, and 164,983 per acre in plot 3. It is also noticeable that the numbers of *Tipulidae* larvae and *Hepialidae* larvae are the same for both plots.

Other groups showed considerable difference in numbers between the two plots. *Diplopoda* occurred at the rate of 1,367,002 per acre in plot 2 and 595,959 per acre in plot 3, while *Trichocera* larvae occurred at the rate of 367,002 per acre in plot 2, but only at the rate of 23,567 per acre in plot 3, and *Chironomidae* larvae, which were found at the rate of 515,151 per acre in plot 2, were only found at the rate of 26,936 per acre in plot 3.

Most of the other groups occurred in somewhat greater numbers in plot 2: only one or two groups were found to be more plentiful in plot 3. Amongst the latter were the *Cecidomyidae* (larvae), 212,121 per acre in plot 3 and 195,286 per acre in plot 2, and the *Chilopoda*, 215,488 per acre in plot 3 and 208,754 per acre in plot 2, although the differences in these cases are not large enough to be of importance.

The equal or almost equal numbers of *Elateridae*, *Tipulidae* and *Hepialidae* larvae appears to show quite clearly that the continued use of farmyard manure does not cause an appreciable increase in the numbers of these injurious species although this manure appears to introduce or attract the injurious *Diplopoda* and certain non-injurious species such as *Trichocera* and *Chironomidae* larvae, which probably are of some service in helping to open up the soil.

11. DISTRIBUTION IN DEPTH.

The depth at which the different organisms occurred was of considerable interest, and the samples were taken in five separate layers in order that their distribution might be accurately determined. This distribution was considerably affected by the ploughing of the plots, but seemed to be very little influenced by the operations of cultivation, harrowing and drilling.

In taking a sample of soil it was usually quite clear to what depth the ploughing had affected the soil, and as a rule a distinct change in the character of the soil was noticed in the fourth layer, taken between the five and seven-inch levels.

Of the total number of insects present, taking the whole period of the investigation, in plot 2, 78·7 per cent., and in plot 3, 50·3 per cent., occurred in the first two layers of soil, that is, between the surface and a depth of three inches. The percentages at the different depths were, for the manured plot: I 51·5; II 27·2; III 11·0; IV 6·4; V 3·8; and for the control plot: I 25·3; II 25·0; III 33·0; IV 11·1; V 5·5. Taking only the period from the commencement of the investigation in February until the plots were ploughed on October 13th, the percentages at the different depths were, for the manured plot: I 58·0; II 27·7; III 9·6; IV 2·5; V 2·2, and for the control plot: I 26·0; II 25·0; III 33·9; IV 10·2; V 4·8. Similarly, from the time of ploughing to the end of the investigation (October to January), the percentages at the different depths were, for the manured plot: I 8·7; II 24·3; III 20·0; IV 32·3; V 15·0, and for the control plot: I 16·6; II 24·0; III 22·2; IV 22·2; V 14·8.

It must be borne in mind, in comparing the percentages in the uppermost layer with those in the other layers, that the volume of soil in this top layer was considerably less than in the other layers, as it consisted of the soil between the surface and a depth of one inch below the surface only, while the remaining layers consisted of the soil for a depth of two inches.

Most groups of insects, etc., considering the period of the investigation as a whole, occurred in the largest numbers in the second layer, with a rather lower percentage in the first. The third usually contained a distinctly smaller percentage than the second, quite commonly being from one-half to one-third the number, while the fourth layer usually stood in about the same relation to the third, the difference being in some cases even greater. The same relation existed again between the fifth and fourth layers. The fact that the figures given above do not coincide with this is due chiefly to the distribution of the ants, which occurred on two occasions in large numbers, owing to the sample containing part of a nest. One of these nests occurred in the first layer of a sample from the manured plot, and the other in the third layer of a sample from the control plot, before the plots were ploughed.

In Fig. 7 the *Formicidae* have been omitted. This diagram indicates very clearly that the *Insecta*, "*Myriapoda*" and *Oligochaeta* (*Terricolae*) probably penetrate to a greater depth than nine inches.

A few groups showed noticeable variations from the above general rule. The *Acarina*, *Cecidomyidae* (larvae), *Chironomidae* (larvae) and *Trichocera* (larvae) were found to occur in much larger proportions in the upper layer than in the second, and very few occurred below the five-inch level. With the *Symphyla* the usual proportions per layer were practically reversed, much the greatest proportion of this group occurring in the fourth and fifth layers.

After the plots had been ploughed the effect of the ploughing on some of the groups of invertebrates was very clear for some time. Taking the numbers of *Collembola* for example, from the beginning of the investigation in February to the time of ploughing in October, the percentages in the five layers were: I 29·0; II 44·0; III 19·8; IV 3·0; V 4·1 in the manured plot, and I 28·6; II 46·8; III 16·1; IV 4·6; V 3·6 in the control plot.

For the period from the time of ploughing to the end of the investigation, the percentages were: I 2·1; II 12·2; III 20·1; IV 44·6; V 20·9 in the manured plot, and I 14·3; II 14·3; III 28·6; IV 28·6; V 14·3 in the control plot.

In the case of the *Elateridae* larvae, taking the whole period of the investigation, the percentages at the different depths were, in the manured plot: I 1·7; II 18·6; III 20·3; IV 32·2; V 27·1; and in the control plot: I 12·2; II 18·4; III 26·5; IV 32·6; V 10·2. Taking only the period from the commencement of the investigation to the time of ploughing the percentages at the different depths were, for the manured plot: I 2·8; II 30·5; III 27·8; IV 22·2; V 16·7; and for the control plot: I 11·6; II 20·9; III 27·9; IV 34·9; V 4·7. After the plots had been ploughed, taking the period from the time of ploughing to the end of the investigation, the percentages at the different depths were, for the manured plot: I nil; II nil; III 8·7; IV 47·8; V 43·5; and for the control plot: I 16·7; II nil; III 16·7; IV 16·7; V 50·0.

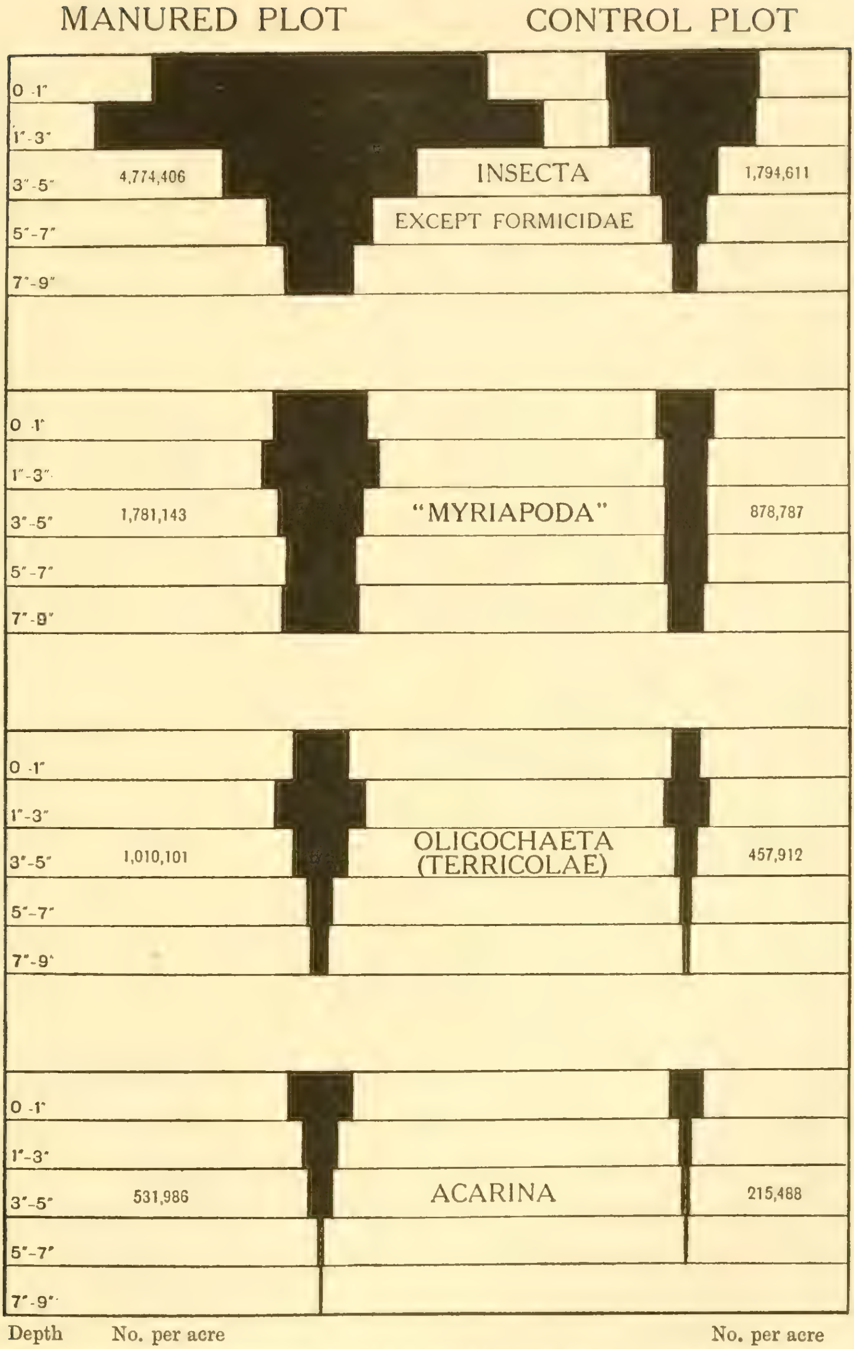


Fig. 7. Distribution in depth of the more important groups in the manured and control plots.

Effects of a similar nature due to the ploughing were observed in some other groups, while with others, such as the *Acarina*, the effect was very little marked, as they appeared to regain the upper layers after being buried by the plough.

Although the percentage at the different depths varied somewhat between the two plots, the general distribution of the insects, etc., was very little different in one plot from that in the other.

No seasonal variation in the distribution in depth of the soil fauna was observed.

12. COMPARISON WITH SOIL FAUNA OF PASTURE LAND.

It is not possible to compare very fully the soil fauna found in the present investigation with that previously found in the examination of permanent pasture (10) owing to the considerable difference in the conditions under which it was existing. The localities in which the work was carried out are widely separated, being in Hertfordshire and Cheshire respectively, and the soil and weather conditions differ considerably.

In pasture land few insects were found at a greater depth in the soil than two inches, and none at a greater depth than six inches. The depth to which insects penetrated into the soil was considered to be chiefly influenced by four factors—depth to which their particular food occurs; aeration; moisture; and temperature of the soil. It was shown that in permanent pasture these four factors all tended to restrict the insects to the superficial layers of soil.

In the present instance these four factors influence the fauna differently, owing to the field being under cultivation. The periodical turning over and stirring of the soil makes it fairly certain that the soil, to the depth to which the implements of cultivation penetrate, will be fairly uniform in composition, and the aeration and drainage of the soil will be more favourable owing to its greater looseness.

In arable soil the conditions are thus much more favourable to deeper penetration by the insects. The number of insects in the control plot is less than was found in the pasture (3,586,088 per acre), but the number in the manured plot is considerably greater.

13. RELATION OF SOIL FAUNA TO SOIL NITROGEN.

In order to determine the importance of the soil fauna as a reserve and source of nitrogen, the nitrogen content of several groups of insects, etc., was estimated, and from these figures it is possible to obtain an estimate of the amount of nitrogen in the whole fauna.

The nitrogen content of the following groups was obtained: *Elateridae* larvae, *Collembola*, *Formicidae*, *Oligochaeta* (*Terricolae*), *Myriapoda* and *Oligochaeta* (*Limicolae*), the percentage of nitrogen in the dry weight being: *Elateridae* larvae 10·65 per cent.; *Collembola* 11·18 per cent.; *Formicidae* 10·92 per cent.; *Oligochaeta* (*Terricolae*) 9·4 per cent.: *Myriapoda* 4·88 per cent.; *Oligochaeta* (*Limicolae*) 6·26 per cent.

The total weight of nitrogen per acre contained in the bodies of the above groups in the manured plot is approximately: *Elateridae* larvae 206·0 gm.; *Collembola* 8·5 gm.; *Formicidae* 306·6 gm.; *Oligochaeta* (*Terricolae*) 4626·0 gm.; *Oligochaeta* (*Limicolae*) 97·0 gm.; *Myriapoda* 1864·9 gm.

Assuming that the remaining insects are of the same average nitrogen content, the total nitrogen of all the insects in an acre of the manured plot is 687·7 gm.

The nitrogen contained in the *Oligochaeta* (*Terricolae* and *Limicolae*) and *Myriapoda* is 6587·9 gm. per acre of the manured plot. These groups include 6,400,668 of the 7,373,730 invertebrates other than insects, the remaining 973,062 consisting chiefly of *Arachnida*, with some *Isopoda* and a few *Gastropoda*. Assuming their nitrogen content to be the same as that of the same number of insects, it would be 74·0 gm. giving a total of 6661·9 gm.

The total nitrogen of the fauna of an acre of the manured plot is thus 7349·6 gm. or 16·2 lbs.

In the control plot the nitrogen contained in the bodies of the same groups is: *Elateridae* larvae 169·0 gm.; *Collembola* 2·4 gm.; *Formicidae* 71·5 gm.; *Oligochaeta* (*Terricolae*) 2128·0 gm.; *Oligochaeta* (*Limicolae*) 21·4 gm.; *Myriapoda* 920·1 gm.

Again assuming that the remaining insects are of the same average nitrogen content, the total nitrogen of all the insects in an acre of the control plot is 313·3 gm.

The nitrogen contained in the *Oligochaeta* (*Terricolae* and *Limicolae*) and *Myriapoda* is 3069·5 gm. per acre of the control plot.

These groups include 2,131,311 of the 2,478,112 invertebrates other than insects in an acre of the manured plot. Assuming that the remaining 346,801 invertebrates have the same nitrogen content as the same number of insects, their nitrogen content is 26·4 gm.

The total nitrogen contained in the bodies of the fauna of an acre of the control plot is thus 3409·2 gm. or 7·5 lbs.

These amounts of nitrogen are equivalent to the nitrogen contained in 103·6 lbs. and 48·0 lbs. of nitrate of soda in the manured and control plots respectively.

It appeared possible that the introduction of insects, etc., in an application of farmyard manure, and their subsequent death and decay with gradual liberation of nitrogen, might account for the effects of an application of farmyard manure being noticeable for a considerable time afterwards. The quantity of nitrogen contained in the fauna seems, however, to be too small to be of great importance in this way, even although the manured plot in this case had received farmyard manure annually for 77 years.

Although the bodies of the invertebrate fauna of the soil contain quite an appreciable amount of nitrogen, there can scarcely be any loss or gain of nitrogen due to them. The *Oligochaeta*, *Myriapoda* and other groups which live and die in the soil, eventually return to it, at their death, all they have taken from it. Although winged insects may leave a plot in which their larvae have fed, this is probably balanced by other insects migrating to the plot and dying there, whose larvae have fed elsewhere.

14. THE FUNCTION OF THE INVERTEBRATE FAUNA IN THE SOIL.

Since the work of Darwin(1) and others(6), (14) the importance of the earthworms in the soil has been widely recognised, the uniformity and loose texture of the surface soil being attributed largely to them. By means of their burrows air and water are enabled to penetrate the soil, and their habit of drawing leaves, blades of grass and other vegetable remains into their burrows adds to their importance.

A considerable proportion of the damage done to land by floods is considered to be due to the flooding out of the earthworms, so that the surface soil remains compacted and vegetation languishes until a new immigration of earthworms has restocked the soil.

Some authors(3), (6), (7), (13) consider that, in addition to the mechanical work of loosening the soil and assisting aeration and drainage, the earthworms, by the passage of considerable quantities of soil through their bodies, render the mineral substances more readily available for plants. On the other hand, the results of other experiments have tended to disprove this theory(12).

It has also been stated that by following the burrows of earthworms, the roots of plants are able to penetrate to a greater depth than would otherwise be the case, although this is denied by other workers(2).

The work of insects, insect larvae and other invertebrates in the soil is probably similar to that of the earthworms(7) in assisting in the aeration and drainage of the soil. Since they pass smaller quantities of soil through their bodies than in the case of earthworms, they probably do not affect the soil to the same extent.

Kostitcheff, in his work on the Russian "Black earth" (8), states that the action of worms and insects in the soil is of great importance in assisting in the breaking down of vegetable matter and the formation of humus. In damp places where worms and insects are unable to live, the vegetable matter is broken down very much more slowly, and peat, in which the vegetable matter still retains a certain amount of structure, is formed instead of an amorphous humus. He does not agree with Darwin with regard to the importance of earthworms in bringing soil from the lower levels to the surface.

In experiments carried out on earthworms, millepedes and *Sciara* larvae Kostitcheff (9) found that they had little effect in accelerating the decomposition of dead leaves, but he considers that after being once passed through the animal, the material is then acted on by fungi and bacteria, and again made available as food for the worms and insects, and in this way the vegetable matter is eventually completely broken down.

Darwin estimated that earthworms brought to the surface of the soil, in their "casts," sufficient earth to form annually a layer 0·2 inch in depth, or dry earth weighing ten tons per acre, and that in 50 years the upper ten inches of soil is completely turned over by them.

Hensen, quoted by Darwin, calculated that there were 53,767 earthworms in an acre of garden soil, and found open burrows to the number of 196,020 per acre, although Darwin states that he has seen them much more numerous. Hensen estimated that there would be half as many earthworms in an acre of cornfield as in garden soil. Darwin, who obtained the number and weight of the "worm-casts" over certain areas, did not give any relation between the number of "casts" and the number of worms present.

In the present investigation the numbers of worms found, 1,010,101 and 457,912 in the manured and control plots respectively, are very much above Hensen's estimates.

SUMMARY.

1. Samples of soil were taken from two of the plots at the Rothamsted Experimental Farm and all insects and other invertebrates were recorded together with the approximate depths at which they occurred.

2. One of these plots (plot 2) has received 14 tons of farmyard manure per acre per annum since 1843; the other (plot 3) has received no manure of any kind since 1839. This difference in treatment had a very marked effect on the number of insects present.

3. Twenty-three samples of soil were examined from each plot, each sample being a cube 9 × 9 × 9 inches. The soil in each sample was removed in five layers, so that it was possible to determine the approximate depth at which the specimens occurred.

4. There were, in round numbers, 15,100,000 invertebrates per acre, of which 7,720,000 per acre were insects, in plot 2, and 4,950,000 invertebrates per acre, of which 2,470,000 per acre were insects, in plot 3.

5. The greatest number, both of insects and of other invertebrates, occurred in the upper three inches of the soil, but some species were found in larger numbers at a greater depth, the greatest number of *Elateridae* larvae being found at a depth of five to seven inches, and of *Symphyla* at a depth of seven to nine inches.

6. Some species, such as the larvae of *Chironomidae* and *Trichocera*, were practically confined to the plot which had received farmyard manure, plot 2, while other species, such as the *Collembola*, *Onychiurus ambulans* and *O. fimetarius*, although they occurred in both plots, were considerably more numerous in plot 2.

7. Injurious insects, such as the larvae of *Elateridae*, *Tipulidae* and *Hepialidae*, appeared to be little affected by the different manurial treatment of the two plots, and occurred in practically equal numbers in the two plots.

8. Although 198,653 and 164,983 *Elateridae* larvae per acre occurred in plots 2 and 3 respectively, they did not produce any appreciable effect on the crop.

9. An attempt was made to estimate the amount of nitrogen contained in the bodies of the soil fauna, and it was found to be 7349·6 gm. or 16·2 lbs. and 3409·2 gm. or 7·5 lbs. in plots 2 and 3 respectively. It is unlikely that there is any appreciable loss of nitrogen from the soil due to the migration of winged members of the fauna.

10. The worms, insects and insect larvae are beneficial in loosening the soil and facilitating aeration and drainage.

11. The net results of these observations show that, although the introduction of farmyard manure greatly increases the invertebrate population of the soil, the latter organisms are saprophagous and are not directly injurious to the growing crop. Such injurious organisms as are present occur in approximately equal numbers whether the land be

manured or not. The most notable exception to this generalisation is met with in the *Diplopoda*, whose numbers are increased by about 200 per cent. in the manured plot.

REFERENCES.

(1) DARWIN, C. (1881). *Vegetable Mould and Earthworms.* London, John Murray.

(2) DJEMIL, — (1898). *Ber. Physiol. Lab. Vers. Halle.* (Referred to by Hilgard (7).)

(3) DUSSERRE, C. (1902). Influence des vers de terre sur la composition chimique du sol arable. *Ann. Agric. de la Suisse*, III. 25–28.

(4) FOWLER, C. (1889). *British Coleoptera*, III. 304.

(5) HAMILTON, C. C. (1917). The behavior of some soil insects in gradients of evaporating power of air, carbon dioxide and ammonia. *Biol. Bull.* XXXII. 159.

(6) HENSEN, M. (1877). Die Thätigkeit des Regenwurms für die Fruchbarkeit des Erdbodens. *Zeitschr. Wiss. Zöol.* XXVIII. 354–364. (Account in *Nature*, XVII. 1877, 18–19.)

(7) HILGARD, E. W. (1914). *Soils.* Macmillan Company, New York.

(8) KOSTITCHEFF, P. (1886). *Potschvi tschernozemskoï oblasti Rossii.* Petrograd (Abstract in *Ann. Sci. Agronom.* II. 1887, Les terres noires de Russie, 165–191.)

(9) KOSTITCHEFF, P. (1889). Recherches sur la formation et les qualites de l'humus (Translation). *Ann. Agronom.* XVII. 1891, 17–38.

(10) MORRIS, H. M. (1920). Observations of the insect fauna of permanent pasture in Cheshire. *Ann. App. Biol.* VII. 2 and 3.

(11) MORRIS, H. M. (1922). On a method of separating Insects and other Arthropods from soil. *Bull. Ent. Res.* XIII. 197.

(12) RUSSELL, E. J. (1910). The effect of earthworms in productiveness. *Journ. Agric. Sci.* III. 1908–10, 246–257, 2 figs.

(13) STEGLITZ, — (1911). *Fühling's Landw. Ztg.* LX. No. 15, 538–542. (Abstract in *Expt. Sta. Rec.* XXV. 1911, 817.)

(14) WOLLNY, — (1890). *Forsch. Agr.* 382. (Referred to by Hilgard (7).)

(*Received April 3rd*, 1922.)

ON THE LIFE HISTORY OF "WIREWORMS" OF THE GENUS *AGRIOTES*, ESCH., WITH SOME NOTES ON THAT OF *ATHOUS HAEMORRHOIDALIS*, F.[1]

PART III

BY A. W. RYMER ROBERTS, M.A.

(*Zoological Laboratory, Cambridge.*)

(With 1 Text-figure and Plates XIII, XIV.)

AGRIOTES SPUTATOR, L.

OF the life history of this species as distinct from that of other members of the genus, not much is as yet known. Köllar in 1837 referred to the larva as feeding on lettuces and describes it as being "light yellow, from six to seven lines long, of the thickness of a pigeon's quill." Curtis(5) (p. 167) and other writers of the nineteenth century seem to have taken their accounts of the species from Köllar, whose details are so very meagre. Adrianov(1), however, as has already been mentioned, obtained the ova and young larvae in Russia in 1914. But he does not appear to have grown the larvae for more than a year and his description of the experiments he made provides no means of distinguishing this larva from others of the same genus.

My own attempts to breed this species from the egg have, from one cause or another, not been very fortunate, though I have obtained ova from my breeding pots in three separate years. The longest-lived brood did not quite survive two years (1916–1918), but from it a few points have at least emerged; firstly, that the rate of growth of the larva within the time named was almost the same as that of *A. obscurus* of the same age, and secondly particular features of the structure have been observed, providing the link connecting the young larvae with older larvae which were taken in the field and from which beetles were bred.

In regard to the first point, only two larvae were obtained after the second winter, but if these can be considered to be of normal size, as is probable, and if their future rate of growth would have corresponded with the past rate, then it appears that there is but little difference in

---

[1] A grant has been received for publication of this paper.

length between *A. obscurus* and *A. sputator* at the same age. Now the larva of *A. sputator* is full-fed when of a length of 16–17 mm. and assuming that its rate of growth is the same as that of *obscurus* it should attain this size by the end of its third year of life. Allowing then for the period at the end of the larval stage, during which but little increase in length takes place, it may be concluded that pupation occurs during the fourth year and that the mature beetle emerges four years after the hatching of the egg, or one year less than the time taken in the life cycle by *A. obscurus*. We have as yet little accurate information as to the duration of the life cycle in other species of the genus, but Xambeu states(12) that *A. sordidus* occupies only one year in the larval stage, while Hyslop found(6) that *A. mancus*, an American species, in the northern United States of America, pupated after three years. It seems probable, therefore, that the duration of the life cycle varies much between different species of the genus and the evidence so far is in favour of *A. sputator* accomplishing it in one year less than *A. obscurus*.

Other points in the life history coincide closely with those of *A. obscurus*, though there may be differences not yet observed. The larvae of both moult twice in the year, they pupate at the same time and the beetle emerges from the pupal condition also at the same time. Köllar says that the duration of the pupal stage is only fourteen days. This has not been verified and may perhaps not apply to the climatic conditions of this country.

No difference in regard to choice of soil has been observed, though *A. sputator* appears to require milder conditions, being comparatively scarce north of Cheshire and Norfolk and rare in Scotland, while it is not known in Ireland. On the continent of Europe also, though its range generally coincides with that of *A. obscurus*, it becomes rather scarce in the centre of Sweden (Thomson) and in Finland (du Buysson), while *A. obscurus* occurs as far north as Lapland.

In regard to the morphology of the larva certain external features, specified in detail later on, have emerged from comparison between the two species, differentiating them from one another. In the first instar, at least, the characters of this species separate it also from *A. acuminatus*, Steph. (*sobrinus*, Kies.). It has not yet been possible to compare it with *A. lineatus*, L. and *A. pallidulus*, Ill. The first-named must, however, from Beling's description, closely resemble *A. obscurus*, while *A. pallidulus* according to the same author seems to lack the sensory pits on the 9th abdominal segment and to resemble rather the larva of *Dolopius marginatus*.

Ovum.

Generally broadly ovoid but varies considerably in both shape and size. Average dimensions of ten ova ·54 mm. × ·43 mm. and therefore slightly smaller than those of *A. obscurus*. One ovum found was almost bean-shaped and measured ·475 × ·43 mm. In this species also the shell is transparent and almost smooth, the whole appearing to be milky white from the colour of the contained yolk and embryo.

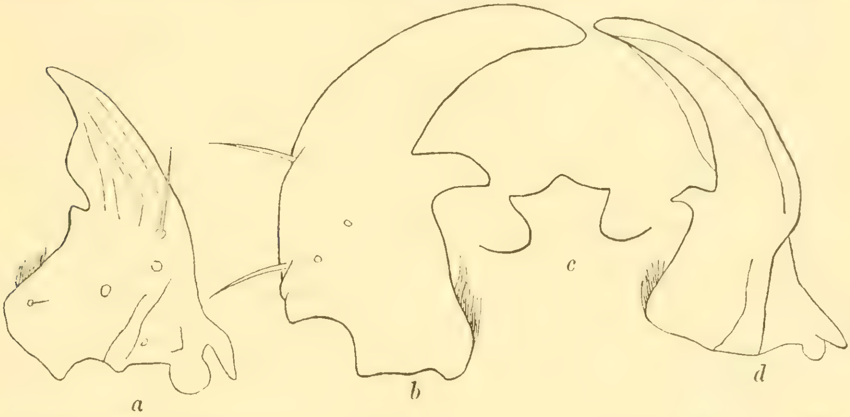
First Larval Instar.

In general appearance the larva is extremely like *A. obscurus* at the same age. The average length during the first day after hatching is just under 2 mm. (1·9), ranging from 1·25 to 2·25 mm. in a dozen specimens; the breadth across the prothorax about ·25 mm. The ventral surface is flat, the dorsal arched, but less so than in older larvae. Colour milky white. It is difficult to see any material difference in the sculpture of the dorsal surface in preserved specimens, but in life the young *sputator* is a trifle more rugose and punctulate.

The head is about equally long and broad, measuring the length from the base of the mandibles to the occiput and the breadth across the broadest part, a little anterior to the middle. It is longer than either the meso- or meta-thorax. As in *A. obscurus*, the mandibles (Text-fig. 1 *a*) are brown at the apex and broader in proportion to their length in the first than in the final instar. The nasale or clypeal process is represented by an entire rounded projection above the mouth. Beneath this, traces of the sub-nasal process are visible, usually as a minute notched process at the base of the nasale, with one or two smaller rounded thickenings of the chitin beyond the lateral margins of the nasale. In the antenna, the third or supplementary segment is longer than the conical ventral process at the apex of the second segment, but much less so than in mature larvae. At this stage, it is also longer in proportion to the whole antenna than in older larvae.

Of the setae with which the tergites are furnished, the posterior row is, as usual, longer than the anterior, but they are not so long as the segments to which they belong. Those of the two rows on the prothorax, anterior and posterior, are of about equal length. The setae of the head and also those surrounding the cauda are shorter than these. Subsequent measurements of the setae of *A. obscurus* at the same stage show that the relative proportions previously given (Pt. II, p. 195) must be amended, those on the abdominal segments being the longest, while those surrounding the cauda and those of the head are considerably shorter, as in *A. sputator*.

The shape of the spiracles at this stage is very variable and does not give any certain means of separation of the species, though the number of teeth on either side of the orifices has been found to be less in *sputator* than in *obscurus*. In the former these number five in the thoracic, four or five, generally four, in the abdominal spiracles. As in *obscurus* the orifices of the spiracle frequently appear to be separately margined by a raised border, most evident at the sides, very fine behind and lacking in front. In reality, however, as may be seen under a high-power objective, the margin is patterned on the surface much as in older larvae.



Text-fig. 1. (*a*) *Agriotes sputator*, L. Mandible of larva in first instar. Magn. cir. × 700. (*b*) *Athous haemorrhoidalis*, F. Mandible of larva in late instar, seen from above. (*c*) *Athous haemorrhoidalis*, F. Clypeal process or nasale of larva. Magn. cir. × 130. (*d*) *Agriotes acuminatus*, Steph. Mandible of larva in first instar. Magn. cir. × 770.

The median area of the septum between the two orifices is lighter in colour and less strongly chitinized but it is in like manner furnished with a corrugated pattern, corresponding to the teeth at the sides of the orifice. The ventral orifice is most frequently smaller than the dorsal one, though considerable variation in size and shape occurs.

Towards the apex of the 9th abdominal segment the same constriction is apparent in both this species and *A. obscurus* at hatching, but disappears later during the first instar. It may be no more than a coincidence, but among the specimens examined the margins of the sensory pits on the 9th abdominal segment are usually coloured brown at or shortly after hatching, whereas in *obscurus* the margins are only to be delimited with difficulty until a much later age.

The cauda, though colourless at first, later becomes slightly tinted with yellow. Its shape affords a slight means of differentiation, for while that of *obscurus* is blunt, it is distinctly pointed, though quite short, in *sputator.*

THIRD INSTAR.

In the early part of the third instar the larva is about 6·5 mm. in length (nearly the same as *A. obscurus* at the same age), and of a pale yellow colour, though this appears to vary somewhat with the individual. In section it is considerably more rounded than specimens in the first instar, but is slightly flatter on the dorsal and ventral aspects than at the sides. In general the larva may be distinguished from that of *A. obscurus* by its coarser punctuation and by its longer and proportionally narrower spiracles.

The head is rather smooth and its setae of the posterior are longer than those of the anterior row. Length of segments of the antennae as 25:13:21, taking the basal, second and supplementary segments. Eyes situated in a line with the anterior pair of setae and behind the antennae. The mandibles appear to be somewhat sharper-pointed and more curved on the outer margin than in *A. obscurus* at the same age. The nasale is distinctly tridentate, with the middle tooth extending considerably further forward than the two lateral teeth. The sub-nasal process is not well defined: in one specimen examined it consists of five rounded teeth borne in an almost straight line at the base of the nasale, the middle tooth being a little more prominent than the rest.

The tergites are coarsely punctured with irregularly-shaped punctures, while the anterior margin of each of the abdominal tergites 1–8 bears a fine and close granulation, which extends backwards as far as the spiracles. This granulation is also present on the 9th abdominal tergite, though the remainder of the tergite is less strongly punctured than that of any other abdominal segment. It is absent from the pronotum, but present on the meso- and meta-notum, where is extends to the anterior row of setae.

The sternites are more sparsely punctured than the tergites but bear a few punctures and also a few somewhat irregular transverse rugae. The posterior margin of the prothoracic sternite behind the coxae and the whole of the meso- and meta-thoracic sternites bear fine granulations.

All the setae are yellowish. Those of the pronotum are about equal in length as between the anterior and posterior rows, while in the first eight abdominal segments the posterior row is the longer. None is as long as the segment to which it belongs. The ventral setae are short.

The spiracles are now distinctly elongate, the ventral orifice being often a little shorter than the dorsal. They are longer and proportionally narrower than those of *A. obscurus* at the same age. At either side of each orifice the teeth or corrugations number 14 to 16 in the thoracic, 10 or 11 in the abdominal spiracles. The marginal rims are brown.

The 9th abdominal segment appears to be narrowed to its apex somewhat more gradually than that of *A. obscurus*, being widest in the region of the sensory pits. These latter are small, round and surrounded with a brown margin. The cauda is short, but a trifle more acute than that of *A. obscurus*. At this stage it is slightly yellower than the surrounding area of the cuticle.

FINAL INSTAR.

In general, the larva closely resembles that of *A. obscurus*, already described in Part II(9). It does not, however, attain the same size, being full fed when of a length of 16–17 mm. and a breadth of 1–1·5 mm. Many specimens are a little darker in colour but this character is not reliable.

Head with a few shallow punctures and short irregular longitudinal striae above and beneath. *Antennae* differ from those of the early stages in having the third or supplementary segment shorter in proportion to the other segments and especially the basal one, the proportion being as 25:9·5:12·75 from basal to third segment. *Mandible* somewhat narrower in proportion to its length than that of *A. obscurus*, but the difference is not very marked. Both species have the posterior one-third on the ventral surface minutely and rather closely punctulate.

The anterior portion of the *cephalic plate*, which overlies the base of the mandible on either side, is in *A. sputator* rather more pointed at the apex than in *A. obscurus*.

By an unfortunate mistake the semi-membranous lining of the *palate* was described in Part II (p. 206) as the floor of the mouth. The "anterior margin" appears to represent a suture found a short distance behind the sub-nasal process, while the tufts of bristles referred to are in reality a portion of those at the anterior margin of the cephalic plate, on its ventral side. The real floor of the mouth is a membranous structure lined with fine hairs and situated behind the base of the laciniae, above (dorsal to) the mentum.

The anterior portion of each *tergite*, with the exception of that of the prothorax, is minutely granulate (Plate XIII, fig. 1*x*). On the meso- and meta-nota and abdominal tergites 1–8 the granulations extend in a transverse band from the intersegmental membrane to a line of minute pores just anterior to the anterior row of setae and to the spiracles in the case of the abdominal segments. On the 9th tergite they extend almost to the anterior margin of the sensory pits.

The main portion of each tergite is, compared with that of *A. obscurus*, distinctly rugose and coarsely pitted with rather deep and irregularly shaped punctures (Plate XIII, fig. 1*c*). The posterior portion of the meso- and meta-thoracic and of the 1st to the 8th abdominal tergites, as well as both anterior and posterior margins of the pronotum, are occupied by similar borders of longitudinal striations as in *obscurus*. The prothorax and 9th abdominal segment are dorsally somewhat smoother than the other segments of the body but have more and deeper punctures than the corresponding segments of *A. obscurus* and also some irregular rugae.

Of the setae with which the tergites are furnished; the longer ones of the two rows on the prothorax are about equally long, but in the case of each of the succeeding segments, up to the 8th abdominal segment, those of the posterior are longer than those of the anterior row. The same proportion in the respective length of the setae applies to *A. obscurus*, though it is not made clear in my description (Pt. II, pp. 200–201). The chaetotaxy of the 9th abdominal segment is also generally the same, though an extra seta near the posterior margin of each sensory pit occasionally present in *A. sputator* and shown in Plate XIII, fig. 1*a*, has not been observed in *A. obscurus*.

In the *pleurite* of the meso-thorax the granulations extend from the anterior margin, along the ventral margin of the spiracle to its posterior end, the dorsal side being smooth. In the epipleurites of the abdominal segments there is also a little granulation at the anterior end of each, the remainder of the surface being punctured similarly to the sternites. On the ventral surface the *prosternum* is almost smooth, but its posterior portion as far as the coxae is granulate and the granulations extend on the posterior side of the coxae themselves for half their length. The whole of the meso- and meta-sterna are granulate and the basal half of the coxae belonging to the same segments have granular areas corresponding to those of the anterior coxae. Similar granulation of areas on the ventral surface of the thorax has been found in *A. obscurus*.

The *abdominal sternites* are more sparingly punctured than the tergites and bear, in addition to the punctures, a number of irregular, more or less transverse, furrows. On the anterior margin of each sternite there is a band of granulations, as on the tergites, extending backward as far as the anterior row of setae. On the 9th abdominal sternite the granulation extends almost to the base of the pseudopod, while that portion of the sternite which lies posterior to the setae bears a few shallow punctures and rugae only.

The *spiracles* (Plate XIII, fig. 1*d*) differ from those of *A. obscurus* in being actually longer (in spite of the smaller size of the larva) and also in being longer in proportion to their breadth. Their sides also are more nearly parallel, the spiracles of *A. obscurus* being widened more considerably anteriorly. Length of the first abdominal spiracle about ·137 mm. and maximum breadth about ·056 mm., while in the larger larva of *A. obscurus* the corresponding measurements are ·125 mm. and ·085 mm. As might be expected the number of teeth or corrugations on either side of the spiracular orifices is also somewhat greater, numbering about 51 in the thoracic and 45 in the abdominal spiracles. Malformation of single spiracles occurs occasionally. Mr Terzi's figure (Plate XIII, fig. 1*e*) clearly shows the nature of the malformation in one specimen while another similar one has also been met with.

Cauda short and generally not very acute: on the average it appears to be slightly sharper than that of *obscurus*.

Apart from the points set out above the description of *A. obscurus* in the late larval stages would serve equally well for this species. As will have been observed, the most salient differences between the two species, apart from size, rest in the sculpture of the cuticle and in the shape of the spiracles. The following comparative table shows the nature of the principal distinctions:

A. obscurus.	*A. sputator.*
Tergites nearly smooth, glossy: bearing shallow furrows, chiefly longitudinal, of variable length: punctures sparse and shallow.	Tergites rather rugose, dull: rugosities irregular, frequently transverse: punctures more numerous, wider and deeper.
Area anterior to spiracles, both dorsally and ventrally, almost smooth.	Area anterior to spiracles, dorsally and ventrally, finely granulate.
Tergite of 9th abdominal segment almost smooth, but bearing a number of shallow furrows irregularly disposed: a few shallow punctures towards the apex.	Tergite of 9th abdominal segment slightly rugose and punctulate: finely granulate anterior to sensory pits.
Spiracles shorter, widest at anterior end.	Spiracles long and narrow; scarcely wider at anterior end.

PUPA.

In general the pupa resembles that of *A. obscurus*, but is smaller, has the prothorax more elongate and differs in several other characters to be specified below.

In length it is about 8 mm. with a breadth of about 2·5 mm. across the thorax. The anterior thoracic spines are attached just above the imaginal eye, which early shows through the integument; they are long and tapering and terminate in a fine brown bristle. The suture between head and prothorax extends from the base of each antenna over the eyes of the adult and is continued ventrally in a semicircle above the vertex of the head.

The prothorax is longer than broad, rounded at the sides, swollen, slightly striated transversely. As in the adult, it is deeper anteriorly and has on the dorsal surface a slight median groove which is deepened posteriorly. The antennae reach to the intermediate pair of legs at the point where the femora are flexed against the tibiae. Their segments are longer than broad, enlarged towards the apex of each segment, except the last, and bear blunt tubercles on either side. The posterior angles of the prothorax are produced into long outstanding spines, fleshy at the base and continued in fine curved brown setae arising from the outer side of the fleshy base. The median spines, situated in many Elaterid pupae one on either side of the medio-dorsal groove, are entirely absent, even the tubercles, visible in *A. obscurus*, being lacking.

Elytral sheaths just reaching to the 5th abdominal sternite, bearing at their apices a small upturned blunt hook. The first abdominal spiracle is concealed beneath the base of the wing-sheath.

Both tergites and sternites of each abdominal segment from the 2nd to the 6th are somewhat sinuate laterally and are produced at their posterior margins into a kind of flange. In life this prolongation of tergites and sternites serves to conceal the spiracles, which are situated in the pleura of each segment. The tergites and sternites of the 7th and 8th segments are scarcely produced laterally and leave the spiracles exposed.

Ventrally, the 7th abdominal sternite is longer than the others and almost paraboloid in shape, though it has in the male pupa, at least, a slight angle at the point where it overlaps the 8th sternite.

The sexual differences are manifest on the ventral surface of the 9th segment as in *A. obscurus*.

The terminal processes arise laterally and project from the body at an angle of some 45°. The basal portion of each is cream-coloured and fleshy, the apical brown and produced into a spine. The spines are neither so long nor so sharply pointed as are those of the prothorax.

AGRIOTES SOBRINUS, KIES. = *ACUMINATUS*, STEPH.

Of this species very little is known. Ova were obtained in 1918 from the soil of a pot within which the beetles had been confined and a few larvae were obtained from these ova. But the eggs laid in the pot appear to have been few and no larvae were reared beyond the first instar. There appear, however, to be no other records of any part of the life history of this insect apart from the adult stage, so that it seems desirable to add what little information is available while dealing with other members of the genus.

In Britain this species is a southern and midland one even more than the last and though generally fairly common in places where it occurs, it is local in its distribution. Abroad it is known over the greater part of the continent of Europe, extending to the Western Caucasus (du Buysson).

The adult is generally referred to as frequenting woody places and flowers, especially those of *Umbelliferae*. Though the beetles are commonly taken in fields and on roadsides away from woods, it is possible that their proper breeding habitat may be in woods, a possibility which has some slight confirmation in the fact that none have yet been bred by the writer from larvae taken in agricultural land.

OVUM.

Outline figures of the ova have already been given (Pt. I, Text-figs. 1 and 2). According to the small amount of material examined, their shape is very variable, but they appear to show a somewhat greater tendency to be pointed at one end than do the ova of *A. obscurus* or *A. sputator*. Average dimensions of six ova ·56 × ·44 mm., the largest measuring ·61 × ·47 mm. In general ovoid, white and somewhat shining, though under the microscope the shell may be seen to be minutely pitted.

Ova were found on the 9th, 10th and 13th July at a depth of $\frac{3}{4}$ to $1\frac{1}{2}$ inch below the surface of the soil. The first larva hatched on the 10th August, so that the incubation period is probably at least a month.

FIRST LARVAL INSTAR.

On hatching the larva is from 2·5–3·0 mm. in length, semi-transparent, milky white to slightly buffish in colour, with the mouth parts conspicuously yellow. Very soon after hatching the gut of larvae confined in a tube with moss was found to be coloured green, indicating that they had eaten the green parts of the moss. In general the larva resembles those of other members of the genus already described, but is more rugose. Other points which may be noted are as follows:

Head about equally long and broad, measured as for other species; longer than meso- or meta-thorax.

Mandible noticeably longer and more incurved than that of either *A. obscurus* or *A. sputator* (Text-fig. 1 *d*). Apex long and sharply pointed, brown, the remainder yellow. Retinaculum longer than in the two species mentioned and distinctly curved backward towards the base. The sub-apical tooth is present as in other *Agriotes* larvae, but is long and narrow, extending as a kind of flange from the retinaculum nearly to the apex.

Nasale or clypeal process consisting of a single robust, somewhat pointed, tooth. Sub-nasal process projecting beyond the mouth cavity, margined with four or five sharply pointed teeth.

In the *antenna* the third or supplementary segment is of equal length to the conical ventral process. Both are longer than the 1st or 2nd segments, which are of about equal length.

Prothorax half as long again as either the meso- or meta-thorax.

Tergites of the body rugose, the rugae being principally transverse but running in all directions. *Setae* in general arranged as in other larvae of the genus, but there are six long, straight, out-standing ones near the apex of the 9th abdominal segment which are somewhat longer in proportion to the rest and stiffer than the corresponding setae of *A. obscurus* or *A. sputator* at this age.

The *spiracles* under a high magnification may be seen to have a distinct, though colourless, margin to the orifices, but they cannot at this stage be distinguished with certainty from those of the other two species.

The *9th abdominal segment* is gradually pointed but constricted before the apex at, and for at least a month after, hatching.

Cauda somewhat similar to that of *A. sputator* but rather more finely tapered. At hatching it is scarcely more coloured than the surrounding cuticle. *Sensory pits*, though present, are very shallow and their margins are not pigmented at first.

ATHOUS HAEMORRHOIDALIS, F.

This species is generally distributed and common throughout the country. The larvae are found in similar situations to those in which *A. obscurus* and *A. sputator* are found, but not in such great numbers. Consequently, though it feeds on the roots of similar plants, the damage done by it, as a species, is small in comparison. Perhaps the greatest damage done by the larva is to potatoes and to tomatoes in greenhouses. The length of the life history is long, probably as long as that of *A. obscurus*. Pupation occurs in August and the beetle emerges after a period of about three weeks. It remains in the soil during the winter, emerging therefrom in May.

OVUM.

An outline figure of an egg was given in Pt. I (p. 133, Fig. 5). The shape is very variable, though always rounded. It may be nearly spherical (·47 × ·42), bean-shaped in profile, or broadly ovoid. Average dimensions of four eggs ·51 × ·41 mm., the largest ·56 × ·43 mm. The shell is transparent, showing the milky-white contents within. Its surface when fresh is clearly granular as seen under a low power of the microscope, the granules being distributed thickly and evenly over the entire surface.

Thirty ova, most of them in a cluster, were found at $\frac{1}{4}$–$\frac{1}{2}$ inch below the surface of the soil on 12th July 1918. Many of them were observed to be advanced in development of the embryo and most hatched on 21–22 July, so that they must have been laid at the beginning of July at the latest.

LARVA IN FIRST INSTAR.

Length at hatching from 1·5 mm. to 2·0 mm. Opaque white, head yellow and with some sign of yellow in the thoracic and 9th abdominal segments.

Head quadrilateral, a little broader than long. *Mandibles* stout, sharply pointed and brown at the apex, with a large, yellow, strongly-recurved retinaculum below the apex on the inner side. *Nasale* or clypeal process distinctly tridented; teeth sharply pointed. *Prothorax* as long as the two following segments taken together. On the dorsal surface it bears 4 setae, the posterior pair scarcely, if at all, longer than the anterior pair. The other segments of the thorax have only one pair of setae, at the posterior end. In the *abdominal segments* there is a transverse row of 4 long setae posteriorly, which are longer than the segments to which they belong. The length of the abdominal segments gradually increases from the 1st to the 8th.

The *9th abdominal segment* is broadest anteriorly and tapers to the posterior end, where it is narrowest. The space is nearly oval and is almost completely closed behind by the inner branches of the processes. This inner branch is tapered to a simple point and is not cuneiform, as in the older larva. The outer branch is yellowish and upturned but blunt at its apex. The two processes are yellowish. The posterior pair of marginal tubercles are alone visible at first, other two becoming visible later in the instar. The flattened disc of the dorsal surface is obvious even in newly-hatched larvae and the sagittal median furrow, with the transverse furrows, are visible in a suitable light beneath even a low magnification. The pseudopod, or anal papilla, is rather large.

LARVA IN LATE INSTARS.

Length 20–22 mm., or according to Beling(2) 24 mm. with a width of 2·6 mm. Biconvex, deep yellow and strongly chitinized on the dorsal surface. Head, prothorax and 9th abdominal segment brownish yellow.

Head transverse, somewhat rounded at the sides. Dorsal surface considerably excavate in the occipital region and apex of the cephalic plate truncated. *Eyes* dark brown, situated posterior to the antennae on either side. A pair of moderately long setae are borne near each of the anterior angles of the head and a longer pair laterally a little posterior to the middle. There is also a longitudinal line of four setiferous follicles on each epicranial plate, midway between the middle and the lateral margin of the head: the most anterior of the setae arising therefrom is long, the remainder very short. *Antenna* longer than that of *Agriotes*, borne on a pale membranous base. The first and second segments brown, with apex of each pale. The third or dorsal supplementary segment is fine, linear and only about one-half the length of the second segment, which itself is about half the length of the first. The ventral process at the apex of the second segment is conical, short and colourless.

Mandible (Text-fig. 1*b*) stout, yellow at the base, dark brown and pointed at the apex, strongly curved inwards; with a rather long, slightly recurved retinaculum, inclined at an angle of about 45° to the apex. There is a penicillus at the base of the inner margin, composed of fine, somewhat wavy, yellow hairs.

Maxillary cardo more distinct than in *Agriotes*, flattened anteriorly at the point of articulation with the stipes and narrowed to a point posteriorly. Sides of the *stipes* nearly parallel and base almost straight and at right angles to them. *Maxillary palps* somewhat long, borne on white membranous palpigers which taper from their bases.

Galea with the first segment much rounded, especially on the outer side.

Lacinia triangular, pointed at the apex and densely clothed with long yellow hairs.

Nasale or clypeal process (Text-fig. 1*c*) brown, broad at the base, transverse, bearing three rather sharp teeth. The median tooth projects forwards, the two lateral ones outwards at an angle of 45°.

Prothorax nearly as long as the meso- and meta-thorax together, shallowly and sparingly punctured, almost smooth. Each succeeding segment to the 8th abdominal segment is progressively more densely punctured and the abdominal segments from 1st to 8th are dorsally progressively more rugose, the first bearing only a few punctures and rugae. Anterior and posterior margins of the prothorax and posterior margins of the other two thoracic, as also the abdominal tergites 1–8, with border of longitudinal striations as in *Agriotes*. *Muscular impressions* of meso- and meta-thorax brown, somewhat raised, forming a slightly obtuse, rounded, angle near the antero-lateral margin. The lateral branch does not quite reach the middle of the segment and the transverse branch extends only half the distance to the medio-dorsal suture. In the first eight abdominal segments the transverse branch from either side meets its fellow at the medio-dorsal line and the longitudinal branch extends posteriorly to the striated border of the tergite. *Legs* rather short and of the same general type as those of *Agriotes*, bearing a few long setae on the inner side and a number of short brown spines arranged in rows on the remainder. Claws rather long, brown, sickle-shaped. Coxae almost globular, strongly chitinized on the inside but membranous on the outer side of each and allowing free movement to the first two segments of the leg within. There is a short oblique dark line in the chitin of the coxa pleurally, from which spring three short stiff spines on the meta-thorax and four on the meso-thorax, though the spines are absent from the prothorax. *Abdominal tergites* 1–8 with a transverse row of 6 long setae near the posterior margin on either side of the medio-dorsal line, and a shorter seta above each spiracle.

Pleurae chiefly membranous, but bearing an elongate sclerite of hard yellow chitin ventral to the spiracle on each. A single long seta is borne at the posterior end of this sclerite, corresponding to those of the tergite and sternite. *Abdominal sternites* 1–8 almost smooth, but with a few fine punctures, yellow and fairly strongly chitinized. Their shape is nearly square but with each postero-lateral margin excavate in proximity to a small, almost triangular sclerite of strong, yellow, chitin. This small sclerite bears a long tapered seta near its posterior margin. A pair of long setae are also situated on the posterior margin of the sternite, one near each lateral angle, and a pair of short fine setae between them. There is a short stiff seta near the anterior angle of the sternite on each side.

The thoracic *spiracles* are situated a little more ventrally than those of the abdominal segments, in the anterior of two isolated rounded sclerites lying between the tergite and sternite on either side of the meso-thorax. Those of the abdominal segments 1–8 are borne in the membrane of the epipleura, midway between the tergite and the elongate pleural sclerite. In shape they are elongate, with a median septum and are little broader anteriorly than posteriorly. Their margins are brown and bear a large number of fine corrugations along their edge. The stigmatic scar is linear, placed transversely close to the anterior margin of the spiracle.

The *9th abdominal segment* (Plate XIV, fig. 2) is flattened on the dorsal surface and is bordered by a raised rim of chitin. It has a median longitudinal furrow, from which three principal tributary furrows branch obliquely forward, connecting with the lateral furrows. These extend in a somewhat indefinite line midway between the lateral margins of the disc and the central furrow. The remainder of the dorsal surface is punctured and furrowed irregularly. At the side of the segment, on the marginal rim, there are four large brownish tubercles, each bearing a long seta from its side, and ventral to these, below the rim, are three or four more similar but smaller tubercles. The shape of the disc is almost circular.

The space between the terminal processes is semicircular anteriorly, but posteriorly it is almost closed by the two converging inner branches of the processes, which are nearly cuneiform. The outer branch of each process terminates in a strong brown, upwardly curved hook, which itself bears a small accessory hook on its inner margin.

On either side of the segment from the anterior margin of the disc, a brownish raised line, resembling the transverse muscular impression of the other abdominal segments, is continued ventrally to the marginal border of striae which surrounds the sternite and pseudopod.

PUPA.

Length of male pupa in natural arched position 10 mm., expanded after fixation 13 mm.; breadth 3 mm. In general it resembles that of *Agriotes obscurus* and bears *spines* at the anterior and posterior angles of the pronotum as well as at the posterior end of the 9th abdominal segment. An additional pair is however borne by this species (and some other Elaterid pupae), one on either side of the median suture at the base of the pronotum. These project outwards and somewhat forwards and are shorter than those at the posterior angles. *Prothorax* both actually and relatively longer than that of *A. obscurus*, with its sides more parallel. *Metathorax* is also longer, bearing a somewhat wide longitudinal suture in its median line, which does not reach either to the anterior or posterior margin of the segment. *Antennae* reach just beyond the intermediate femora. A little below the apex of each antennal segment from the 3rd to the last there is a whorl of small tubercles. *Maxillary palps* rather long and incurved. Apices of *elytral cases* tapering to a fine point and bearing at the apex a short reflexed brownish hook.

The margins of both *tergites* and *sternites* of the abdomen are somewhat more produced than in *A. obscurus*. Dorsal surface minutely and shallowly punctate. Sternite of 7th abdominal segment produced in the form of a triangle, partially covering, and the apex reaching to the posterior margin of, the 8th segment. *Abdominal spiracles* situated in the pleurites, near their anterior margins, thoracic spiracles between the pro- and meso-thorax. In shape they are also like those of *A. obscurus*, as well as in position. The *terminal* pair of *spines* are somewhat short and bear on the inner margin of each a short sharp process in the male and female pupae examined. Sexual organs visible on the ventral surface of the 9th abdominal segment and approximately similar to those of *Agriotes*.

CORYMBITES CUPREUS, F.

This is a mountain-loving species and extends in suitable situations throughout temperate and central Europe to the Caucasus (du Buysson). The form *aeruginosus* appears to be merely a colour variety and is generally found where the typical form occurs. In Great Britain and Ireland it is widely distributed, but is common only in the higher-lying districts. In such localities the larva is commonly found in turf and under stones and, though no records of its harmfulness are known to the writer, it seems probable that minor damage may have been done, since the larva feeds in captivity on the roots of various plants. It is only fair, however, to point out that Xambeu(11) found them feeding on larvae of *Aphodius* and it is possible that both animal and plant food is taken. Other species of the genus are well-known pests of crops, principally in America(6).

The larva apparently moults twice in the year and eventually pupates in an earthen cell in the ground in July or August. It emerges from the pupal condition in about three weeks, but remains in the earth as a beetle during the winter.

Beling(2) has described the larva and pupa of this species under the name of its variety *aeruginosus* and distinguishes it(3) from the very similar larva of *C. pectinicornis* by the stronger punctuation and rugosity of the abdominal tergites.

LARVA.

Length up to 25 mm., breadth across thorax 3·5. Colour above olive brown, with the sides, the ventral surface and usually the posterior margins of the segments yellow. The medio-dorsal suture and the membranous parts of the cuticle white.

Head broader than long, brown above, yellow beneath. Occipital region somewhat excavate and the cephalic plate truncated behind. *Eyes* black. Four setiferous follicles in a line on each epicranial plate, as in *Athous haemorrhoidalis*, but the setae are fairly long. *Mandibles* short and broad, incurved, brown at the apex, with a penicillus at the base on the inner side. A strong tooth (retinaculum) of brownish colour is situated on the inner margin nearer the apex than the base. It is scarcely recurved but in position is inclined somewhat towards the apex. *Hypostome* wider in front than behind, with outer margins nearly parallel. There is an indication of a sub-galea at the base of the triangular lacinia, separated from the palpiger by a more or less distinct suture. *Nasale* or clypeal process composed of a single triangular tooth, rather long and pointed. Sub-nasal process apparently absent.

Prothorax as long as meso- and meta-thorax together, sparsely punctured with shallow punctures and having a few irregular furrows. Meso- and meta-thorax with muscular impressions as on abdominal segments, but shortened, the transverse branch reaching only half way to the median suture and the lateral even more reduced. *Coxae* somewhat similar to those of *Athous haemorrhoidalis*, with a similar oblique row of short spines arising from a linear cleft a little anterior and ventral to them.

Surface of *abdominal tergites* 1–8 sparingly but deeply punctured, chiefly in front. Punctures and rugae increase in number successively on each segment. Anterior setae consist of two short ones, one on either side of the median suture; the posterior row of five longer ones on either side of the median suture, arranged transversely. *Muscular impressions* brown, raised above the surface of the tergite, and together forming nearly a right angle; transverse branch somewhat sinuate and not quite reaching the median suture; lateral branch not quite reaching the posterior row of setae.

Pleurites elongate, narrowed in front and behind. Each bears two contiguous setae, a long and a short one, in line with the posterior row of tergal setae. Ventral to these and situated within the membranous part of the pleura (hypopleurite?) is another seta of medium length. *Sternites* quadrangular, almost smooth, bearing a longitudinal row of three setae along the lateral margin on each side. A single shorter seta is placed between the most posterior of these and the medio-ventral line.

Spiracles rather short, almost pyriform, broader in front than behind; situated within the membrane between tergite and pleurite. Their margins are brown, as also is the transverse scar placed almost immediately anterior to each. The cuticle anterior to the spiracle is corneous for a short distance only.

9th abdominal segment (Plate XIV, fig. 3) above deeply and fairly strongly punctured anteriorly. Disc with four principal furrows; the middle pair, situated one on either side of the middle of the segment, converging posteriorly but not meeting; the outer and longer pair nearly parallel to the lateral margins of the disc. A number of irregular tributary furrows join the principal ones at all parts of their length and sometimes connect them. Margin of the disc raised above its level anteriorly and at the sides in a rim running out into the recurved prong of the outer branch of the terminal process on either side. Inner branches of terminal processes pointed, with the apices all but meeting and enclosing the space between the processes. The space so enclosed is nearly oval. At each side of the disc, just ventral to the marginal rim, are three principal oval brown tubercles, from the side of each of which a long curved seta arises. A number of smaller brown setiferous tubercles are situated ventral to the three principal ones.

The anterior margin of the disc is continued down the sides in a slightly sinuous raised impression almost to the arched striated border separating the tergite from the sternite. A row of about twelve setae of various lengths forms a line at the edge of the striated border surrounding the pseudopod or anal tube.

Mr K. L. Henriksen of the University Zoological Museum, Copenhagen, has kindly given permission for use to be made of his generic table, originally published in the paper so often referred to here. Certain verbal alterations have been made and the table itself altered so as to include as far as possible the British genera of Elateridae, while excluding those unknown to this country. I would here tender my sincere thanks to Mr Henriksen.

LARVAE OF BRITISH ELATERIDAE.

1.	Abdomen soft, whitish	*Cardiophorus*
	Abdomen with terga at least strongly chitinized	2
2.	Submentum triangular. Retinaculum absent	*Lacon*
	Submentum somewhat linear. Retinaculum present	3
3.	9th abdominal segment with a single tooth at the apex or simply rounded	4
	9th abdominal segment ending in two short processes	12
4.	9th abdominal segment simply rounded at the apex	5
	9th abdominal segment ending in a tooth	6
5.	Tergites punctulate. Head convex above	*Sericosomus*
	Tergites densely rugose transversally. Head flattened above	*Ludius*
6.	9th abdominal segment flattened above, angular at sides	*Melanotus*
	9th abdominal segment rounded above, conical	7
7.	Integument without deep punctures or rugae	8
	Integument coarsely punctured	10

8. 9th abdominal segment with transverse rows of large setiferous tubercles, without sensory pits . . . 9
9th abdominal segment without transverse rows of setiferous tubercles, with two sensory pits . . . *Agriotes*

9. 9th abdominal segment with 3 rows of setiferous tubercles . . . *Dolopius*
9th abdominal segment with only 2 rows of setiferous tubercles . . . *Adrastus limbatus*

10. Anal tube situated under the posterior third of 9th abdominal segment . . . *Megapenthes*
Anal tube situated under the anterior third of 9th abdominal segment . . . 11

11. 9th abdominal segment narrowed conically from the base, with transverse rows of large setiferous tubercles . . . *Ischnodes*
9th abdominal segment cylindrical at the base, without tubercles . . . *Elater*

12. The two terminal processes simply bending inwards . . . *Limonius*
The two terminal processes each ending in 2 prongs . . . 13

13. Inner prong of terminal process forming principal branch . . . *Hypnoidus*
Outer prong forming principal branch or equal to the inner prong . . . 14

14. 9th abdominal segment with a more or less deep median furrow . . . 15
9th abdominal segment without median furrow . . . *Corymbites*

15. Nasale ending in a single tooth . . . *Corymbites aeneus*
Nasale ending in 3 teeth . . . 16

16. Space between the two terminal processes small with narrow aperture posteriorly . . . 17
Space between the two terminal processes large with wide aperture . . . *Athous* (pars)

17. Terminal process with inner prong quite smooth and simple . . . *Campylus*
Terminal process with inner prong very rough or projecting forward within the space . . . *Athous* (pars)

REFERENCES.

(1) Adrianov, A. P. (1914). *Rept. on work of Entom. Bureau* (of Kaluga), 1913–1914.

(2) Beling, Th. (1883). *Deutsche ent. Zeits.* xxvii. 270, 293.

(3) —— (1884). *Deutsche ent. Zeits.* xxviii. 207, 208.

(4) du Buysson, H. (1906). *Faune Gallo-Rhenane-Coléoptères,* v. Caen.

(5) Curtis, J. (1860). *Farm Insects.* London.

(6) Hyslop, J. A. (1915). *U.S. Dept. Agric. Bull.* No. 156.

(7) Köllar, Vincent (1840). *A Treatise on Insects injurious, etc.* (Eng. transl.). London.

(8) Roberts, A. W. R. (1919). *Ann. App. Biol.* vi. 116–135.

(9) —— (1921). *Ann. App. Biol.* viii. 193–215.

(10) Thomson, C. G. (1864). *Skandinaviens Coleoptera,* vi. 92. Lund.

(11) Xambeu, — (1912). *Ann. Soc. Linn. de Lyon,* lix.

(12) —— (1913). *Ann. Soc. Linn. de Lyon,* lx.

EXPLANATION OF PLATES XIII AND XIV.

Fig. 1.

Larva of *Agriotes sputator*, L. *a.* Terminal segments. *b.* Cuticle of 9th abdominal segment between the sensory pits. Highly magnified. *c.* Cuticle of 8th abdominal segment along medio-dorsal suture. Highly magnified. *d.* Normal spiracle. *e.* Abnormal spiracle. *x.* Granulations of anterior margin of tergite.

Fig. 2.

Athous haemorrhoidalis, F. Dorsal surface of 9th abdominal segment of larva.

Fig. 3.

Corymbites cupreus, F. Same.

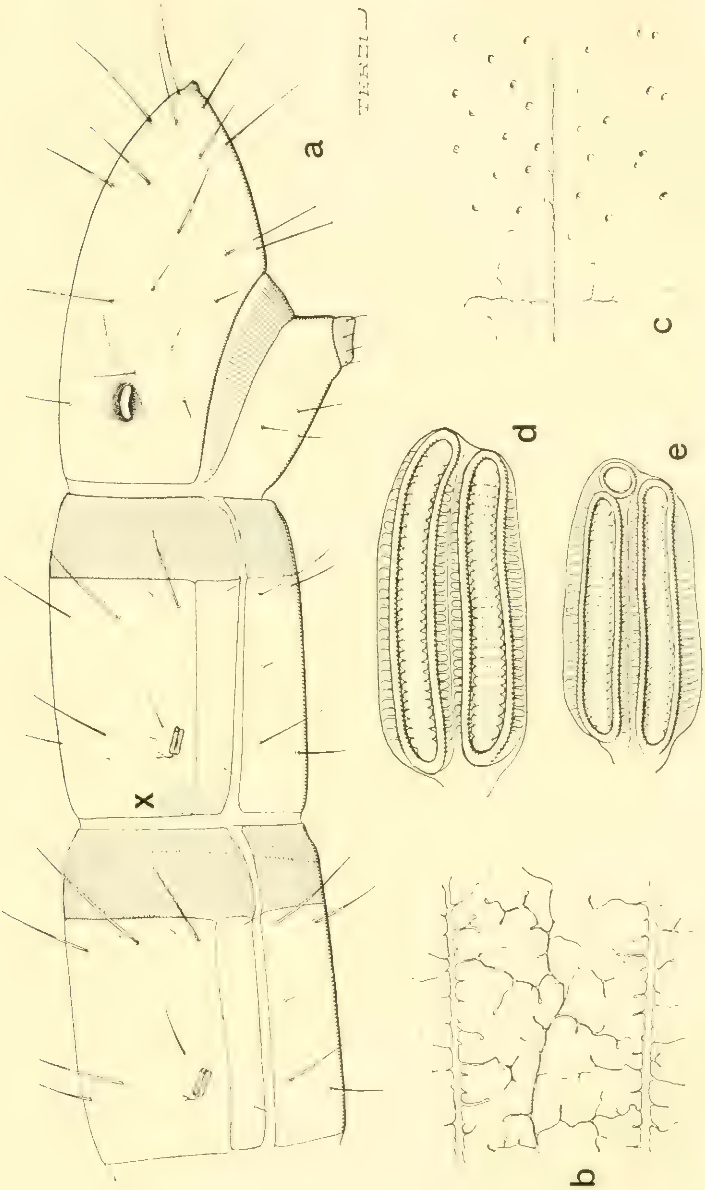


Fig. 1.

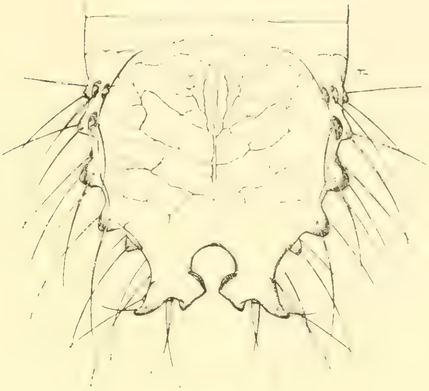


Fig. 2.

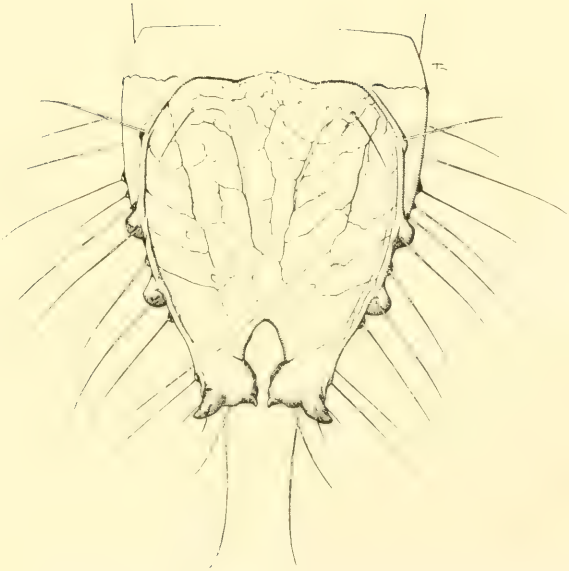


Fig. 3.

THE ACCURACY OF THE PLATING METHOD OF ESTIMATING THE DENSITY OF BACTERIAL POPULATIONS

WITH PARTICULAR REFERENCE TO THE USE OF THORNTON'S AGAR MEDIUM WITH SOIL SAMPLES

By R. A. FISHER, M.A., H. G. THORNTON, B.A.,
and W. A. MACKENZIE, B.Sc.

(*Rothamsted Experiment Station*)

(With 2 Text-figures)

1. Introduction

The accuracy of the estimates of bacterial density, in samples of soil, water, or other material, obtained by the plating method, is only one of many points which arise in the interpretation of bacterial counts. The full interpretation of such data would include a consideration of the divers species that occur on the culture media, and of the forms in which they exist in the soil. The partial or total exclusion of certain forms, such as anaerobes, that require special cultural conditions, must also be considered in a full examination of such data, for a single medium supplies, necessarily, but a single aspect, however comprehensive, of the bacterial flora of the soil. Questions too, as to what is to be considered as the unit of enumeration—the individual organism as it exists in the soil, or possibly groups of such organisms adhering to single particles of soil, and undetached by the processes of sampling and dilution—whatever their importance may be, are not the object of the present investigation.

For if all these inquiries could be answered with certainty and precision it would still remain to be discovered with what accuracy the numerical estimate of bacterial density, obtained from a single set of plates, represented the actual bacterial density in the sample, and in the material from which the sample was drawn.

The question of *accuracy*, therefore, unlike the other elements in the interpretation of bacterial count data, is primarily a statistical question and may be thrown into the characteristic statistical form of the estimation of a population from a sample. Only in peculiarly favourable cases, however, as will be seen more clearly below, could we rely upon an *a priori* mathematical solution.

2. THE PLATING METHOD

The plate method of counting soil bacteria is an adaptation of the plate counting technique, developed by Koch in 1881, applied to the special conditions of soil bacteria.

The process in general consists in making a suspension of a known mass of soil in a known volume of salt solution, and in diluting this suspension to a known degree. The bacterial numbers in this diluted suspension are estimated by plating a known volume in a nutrient gel medium and counting the colonies that develope on the plate. An estimate of the bacterial numbers in the original soil is then made by a simple calculation, the mass of soil taken and the degree of dilution being known.

There are great variations in the details of the method as employed by various workers. These differences concern all the stages in the process and also the nature of the gel medium used in plating. An idea of the extent of this lack of standardisation may be gathered from a paper by Z. N. Wyant(16) in which a number of the variations in technique used by different workers has been collected from the literature.

As an example illustrating the process, however, the technique used at Rothamsted and employed by Cutler in the bacterial count work discussed below, will be described.

Ten grams of the soil sample are placed in 250 gm. of sterile saline solution and shaken for four minutes to obtain a suspension of the soil. 1 c.c. of this suspension is placed in 99 c.c. of sterile saline solution and shaken for one minute to ensure a uniform distribution of the contained organisms. 1 c.c. of this second dilution is placed in another 99 c.c. of saline and shaken for one minute.

Every cubic centimetre of this final dilution will then contain $\frac{1}{250000}$ grams of the original soil sample.

One c.c. of this dilution is then delivered into each of five petri dishes and mixed with an agar medium. After incubation the bacterial colonies on each plate are counted, and the mean of the five parallel counts taken. From this the bacterial numbers per gram of soil are estimated.

The bacterial numbers obtained by the plating method do not represent the total bacterial content of the soil. This is clear from the fact that on no single medium will all the physiological groups of soil bacteria develope. In using this method, however, it is hoped to obtain a standard of bacterial density by which two or more soil samples can be compared. To obtain this result from the method a careful standardisation of the whole technique is essential, in order that those sources of error that cannot at present be eliminated, such as the failure of some organisms to develope on the plates, may be rendered so uniform as to affect the count in a constant manner.

This standardisation must comprise both (*a*) the manipulative portion of the technique involved in making the dilutions, and (*b*) the composition of the medium employed in plating.

In applying results obtained by the method it is necessary to have an estimate of its degree of accuracy, and in order to improve it, some knowledge must be obtained as to which stages in the process are the chief causes of the variation in results.

For the results of the plating method to have their highest possible accuracy, very severe conditions would have to be fulfilled. An imaginary experiment will perhaps serve to make the conditions clear.

If a 10 gm. sample of soil were diluted down to a dilution of 1 gm. in 250,000 c.c., enough material would be provided for $2\frac{1}{2}$ million plates. The result of such an experiment would be of the highest possible accuracy, if one could assume that

(I) Each plate offers the same facilities for development.

(II) The development of any organism is independent of other organisms present.

(III) Development results in only one visible colony.

Since in practice only a few plates are prepared, two additional conditions are involved in the sampling theory.

(IV) Each plate has an equal chance of receiving any organism.

(V) The organisms are distributed independently.

The fulfilment of the first, fourth and fifth conditions depends upon the perfection of the technique employed. The second and third conditions depend definitely on the nature of the organisms, and are only matters of technique in so far as this term may be employed for the choice or elaboration of a medium upon which the organisms, which it is desired to study, fulfil those conditions, and which excludes the interference of those which would fail to do so.

These conditions can to some extent be tested independently. Thus, in a short experiment, where a single batch of medium is used, it is to be expected that the medium in each plate will offer the same facilities for development (Condition I). In a long experiment, however, where a number of different batches of medium are used, this will be the case only if the medium can be accurately reproduced, if, that is, different batches of medium, prepared independently, give significantly the same results. This reproducibility has been confirmed for Thornton's agar medium (Thornton, 1922(11)).

Again condition (IV) would fail if from any cause the dilution was carried out in an irregular manner. This may be tested directly by carrying through the whole dilution process independently with different portions of the same sample. The following experiment is an example of such a test.

Four portions of a sample of Barnfield soil, simultaneously analysed by four different workers (Aug. 14, 1921), gave the following counts:

Table I

	Portion			
Plate	A	B	C	D
1	26	28	31	37
2	30	33	26	32
3	30	32	28	32
4	29	26	32	30
5	32	27	31	26
Mean	29·4	29·2	29·6	31·4

The four sets of plates are indistinguishable from random samples from a single population. The variance estimated as from a single sample of 20 is 8·52, actually less than the mean value for the variance within each set, 9·15. An equivalent test is provided by the correlation between different plates of the same set; this is −·089 ± ·108, negative and quite insignificant. In spite of the fact that the different plates of the same set agree very closely, the variation between the four means is quite insignificant.

Table II

	Portion			
Plate	I	II	III	IV
1	72	74	78	69
2	69	72	74	67
3	63	70	70	66
4	59	69	58	64
5	59	66	58	62
6	53	58	56	58
7	51	52	56	54
Mean	60·86	65·86	64·28	62·86

Equally close is the agreement between the sets of seven plates prepared from four parallel series of dilutions (June 22, 1922), shown in Table II. No trace of differentiation is observable, and the four sets must be regarded as random samples from a single population.

On certain occasions the same point is established by the analysis of simultaneous samples from the same field. An agreement in such cases shows the uniformity in bacterial density of the portion of the field sampled; it also serves to show that no significant differences are introduced by variations in the process of dilution. Thus four simultaneous samples from Broadbalk (Aug. 14, 1921) gave the following counts.

Table III

	Sample			
Plate	I	II	III	IV
1	38	45	43	27
2	32	40	34	41
3	52	45	52	35
4	32	31	55	36
5	40	43	38	45
Mean	38·8	40·8	44·4	36·8

From the whole set of 20 the variance is 56·27, from the four sets of 5, 56·97, not a significantly greater value. The correlations between plates of the same group is + ·014 ± ·108, an insignificant positive value. By the most sensitive tests possible, no differentiation is observable.

There is thus reason to claim that the manipulative technique can be so efficiently standardised that no significant variations in it are detectable, having regard to the variance that occurs between the colony numbers developing on parallel plates from a single final dilution.

Our attention is thus drawn to this variance between parallel plates, which may be due solely to the chance distribution of organisms within the final dilution, or may in addition be influenced by the mutual interference between organisms on the plates, or by the failure of certain organisms to develope into single discrete colonies.

It is therefore necessary, in interpreting the results of the counting technique, to discover the relative importance of these influences, on the colony numbers, and on the variance between them. It is on the experimental evidence as to the actual nature of this variance between parallel plates that our further conclusions will be based.

Nevertheless, the two questions of the reproducibility of the medium and of the equivalence of results obtained by independent series of dilutions made from a single sample, are here insisted upon, because failure in either of these two points would not necessarily affect the agreement between parallel platings, from the same final dilution, which is studied below.

3. THE POISSON SERIES

It was shown by Poisson (1) in 1837, that if a large number of individuals, N, are each exposed independently to a very small risk of an event of which the probability of occurrence in any instance is p, then the number of occurrences, x, in any trial will be distributed according to a definite law, sometimes called the Law of Small Numbers. The distribution of x is found to depend on a single parameter

$$m = pN,$$

in such a way that the probability that the number of occurrences shall be x is given by the formula

$$e^{-m}\frac{m^x}{x!}.$$

It should be noted that x is always a whole number, while m may be fractional; the mean value of x is equal to m, and when m is large the distribution, except for its essential discontinuity, resembles a normal distribution, having its mean at m and the *variance* (the square of the standard deviation) also equal to m.

The importance of the Poisson series in modern statistics was brought out by "Student" (2) in 1907[1], in discussing the accuracy of counting yeast cells with the haemocytometer. Since the chance of any given yeast cell settling upon any given square of the haemocytometer is extremely small, while the number of cells is correspondingly great, "Student" arrived independently at the Poisson formula, as a theoretical result under technically perfect conditions. He was able to show that, in some instances, counts of 400 squares agreed with the theoretical distribution, and that when this is the case the accuracy of the count is known with precision and depends only on the number of cells counted[1].

---

[1] The Poisson Series had been successfully applied by von Bortkiewicz to the annual number of deaths from horse-kick in a number of Prussian Army Corps (*10*). Miss Whitaker's criticism (*8*) of this application is entirely vitiated by her neglect of the variation of random samples.

H. Bateman (1910) (*9*) arrived at the formula for the Poisson Series, as the distribution of the number of α particles, emitted by a film of polonium, which strike a sensitised screen in successive equal intervals of time. The formula was used by Rutherford and Geiger to test the independence of simultaneous emissions. The distribution of 2608 counts shows a general agreement with expectation, though there are discrepancies not easily to be explained by chance. The observations are certainly not adequate, as these authors suggest, as "a method of testing the laws of probability."

The ideal conditions for bacterial counts made by the dilution method, are closely parallel to those found necessary in the case of the haemocytometer. The chief practical difference lies in the fact that instead of 400 squares with only a few yeast cells in each, we have some five plates with perhaps 200 colonies apiece. The agreement of the results with the theoretical distribution cannot, therefore, be demonstrated from a single count. Under ideal conditions the data would consist of a number of small samples from different Poisson series. For this reason as soon as it was suspected that this ideal condition might have been realized in practice, a special investigation of the nature of such samples was undertaken, owing to the importance of demonstrating the substantial fulfilment of the severe conditions laid down in the previous section.

4. PRELIMINARY REDUCTION OF CUTLER'S DATA

When the question of the accuracy of the bacterial counting technique was discussed between the present authors in the spring of 1921, it was decided that the daily observations of bacterial numbers then being carried out at Rothamsted by Cutler would afford a valuable opportunity of studying the variance between parallel plates and its causes. In this choice our investigation was more than fortunate, for no other series of bacterial counts known to us, of which many have been examined, would have gone so far in clearing up the obscurities of the subject.

In conjunction with daily estimations of soil protozoa carried out at Rothamsted from July 1920, daily counts of bacteria were also made in the protozoological laboratory (Cutler(17)). The dilution technique used in this work has been described above. Plates were incubated at 18° C., and counted after five and seven days, the seven day counts only are considered here. Throughout the work the agar medium recently elaborated by Thornton(11) was used. The data thus supply an extensive test of this medium under routine conditions.

When the statistical examination of these data was commenced it was not anticipated that any clear relationship with the Poisson distribution would be obtained; the reduction was designed to determine empirically the relation between the mean bacterial number calculated from any set of plates, and the variability of that set about the mean. Knowing this relation, a probable error could be assigned to each value.

---

[1] Valuable tables of the Poisson Series have been prepared by H. E. Soper(7).

Two statistics were calculated from each set of plates. If x stand for the number of colonies on each plate, and n for the number of plates, the necessary statistics were:

the *mean* $$\bar{x} = \frac{1}{n} S(x),$$

and the *variance* $$v = \frac{1}{n-1} S(x-\bar{x})^2,$$

where S stands for summation.

The values of v, being the estimates of the variance from small samples, were inevitably affected by large sampling errors, which depended upon the number of plates. The whole body of four-plate sets was therefore divided into groups, according to the value of $\bar{x}$. Thus for the two groups of four-plate sets having a mean number of colonies 65–75 and 75–85, the following values of v were obtained:

Table IV

	65–75	
Set No.	$\bar{x}$	v
29	69·75	65·58
33	73·50	27·00
51	68·75	312·25
60	71·50	401·67
128	73·75	60·91
164	72·75	146·25
227	67·50	27·67
241	68·75	8·91
249	67·25	7·58
263	73·25	112·58
272	72·75	52·91
330	70·00	55·33
Mean of 12		106·55
Mean of 10		56·47

Table V

	75–85	
Set No.	$\bar{x}$	v
59	77·00	78·00
89	76·75	142·91
97	84·75	144·25
105	84·50	56·33
149	79·50	77·67
169	84·50	123·67
240	82·25	8·91
273	84·50	48·33
301	84·25	73·91
Mean		83·78

Two facts are apparent from these results (1) the variability of v is so great that accurate values are not obtained from the means of about 10 values; (2) the difficulty of estimating the variance for given values of $\bar{x}$ is still further increased by the occurrence of occasional very large values of v. The values of v in sets 51 and 60 in Table IV are much greater than the other 10 values in the same group. The values of the means obtained by excluding and including these high values are given at the foot of the table.

The first difficulty could be overcome by fitting to the actual values obtained a smooth curve representing the mean v for given $\bar{x}$: before doing so, however, it was thought advisable to exclude as far as possible the exceptional large values. As a rough criterion it was decided to exclude those values which exceeded by more than threefold the mean value of the group. In the larger groups this criterion acted well; in the smaller groups, such as occurred for high and low values of $\bar{x}$, it was necessarily inconclusive, even when account was taken of neighbouring groups. The curve fitting was therefore confined to the region in which the data appeared to be sufficiently abundant.

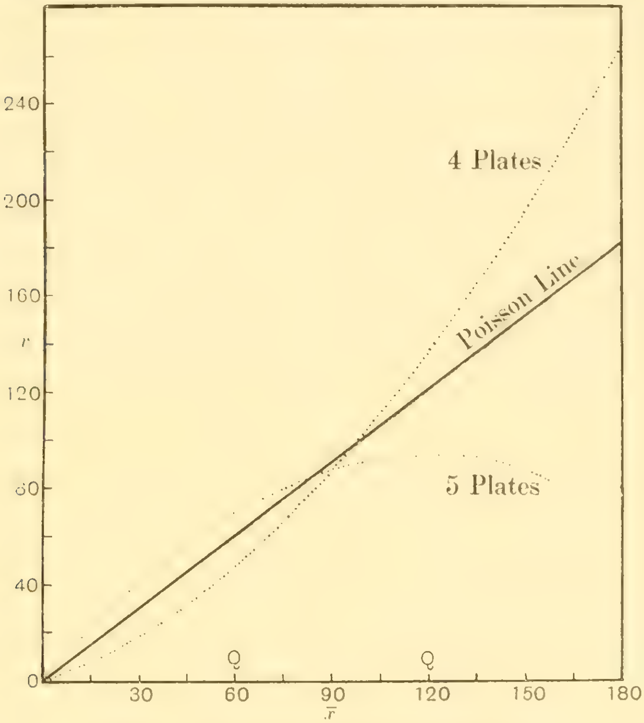


Fig. 1. Smooth curves fitted to Cutler's data.

Curves of the form $v = A\bar{x} + B\bar{x}^2$

(where A and B are two constants determined from the data) were fitted to the four-plate data from $\bar{x} = 0$ to $\bar{x} = 180$, and to the five-plate data from 0 to 160; the curves obtained are shown in Fig. 1.

The straight line, $v = \bar{x}$, represents the relation between the variance and the mean in the Poisson Series. The curves evidently tend to cling closely to this line, especially in the region (60-120) where the data are most abundant. The curves strongly suggested that the departures in these data from the Poisson samples were not, as had been expected, *systematic*, but were due to the *sporadic* occurrence of exceptional sets; the curvature in the smooth curves being perhaps largely due to the crudity of the criterion employed in excluding the exceptions. This view impressed the authors with the necessity of studying the distribution of small random samples from the Poisson Series, with the double object of devising a valid criterion for the recognition of exceptions, and of testing accurately whether or not the remainder were in reality such random samples.

5. Small Samples of the Poisson Series

The study of small samples, essential as it is to the development of adequate statistical methods, has hitherto been practically confined to the normal curve and surface. The following investigation may serve to show, that by taking account of the fundamental properties of those statistics which are derived by the method of Maximum Likelihood, the sampling problems of even discontinuous distributions admit of material simplification.

In a sample from a Poisson Series, the chance of any observation having the value of x is

$$e^{-m}\frac{m^x}{x!},$$

where m is the parameter of the series.

Hence the chance of observing a given series of values $x_1, x_2 \ldots x_n$ is

$$\Delta f = e^{-nm}\frac{m^{nx}}{x_1!\,x_2!\ldots x_n!}.$$

If we estimate m from such a sample by the method of maximum likelihood, we have

$$\frac{\partial}{\partial m}(\log \Delta f) = -n + \frac{n\bar{x}}{m} = 0,$$

so that $\bar{x}$ is the most likely value of m, and in consequence, as Fisher has recently shown(3), it may satisfy the criterion of sufficiency, in which case the distribution of any other statistic, for a given value of $\bar{x}$, must be independent of m.

That this is so may be proved directly; for

$$e^{-nm}\frac{m^{nx}}{x_1!\,x_2!\ldots x_n!}$$

may be put into the form

$$e^{-nm}\frac{(nm)^{n\bar{x}}}{(n\bar{x})!}\cdot\frac{(n\bar{x})!}{n^{n\bar{x}}\,x_1!\,x_2!\ldots x_n!},$$

the first factor represents the chance of obtaining a given value of $\bar{x}$, and the second, which does not involve m, gives the chance that the sample shall show any particular partition of the total, once the total is fixed. The distribution of any statistic which depends upon this partition, must therefore be independent of m, once $\bar{x}$ is fixed. The problem of the distribution of v is therefore susceptible of the great simplification, that we need only consider its distribution for given values of $\bar{x}$, and that this distribution is wholly independent of m.

The distribution of this, or any other, statistic, which depends upon a partition of an integer, must necessarily be discontinuous; when, however, $\bar{x}$ is large, even for small values of n, the number of possible values of v becomes sufficiently great for its distribution to be represented by a frequency curve. This procedure is the more advantageous in that, by the choice of a new statistic, which shall replace v, we can throw the distribution into a form independent of $\bar{x}$, whereas the actual partitions possible in the neighbourhood of equipartition, will necessarily change with the fractional part of $\bar{x}$.

The frequency with which any given partition of the total, $n\bar{x}$, occurs, is in fact the frequency with which any given series of values are obtained when the total is distributed at random into n cells, the expectation in each being $\bar{x}$. It is well known that when this is the case, the statistic

$$\chi^2 = \frac{1}{\bar{x}} S\,(x - \bar{x})^2 = (n - 1)\,\frac{v}{\bar{x}}$$

measures the departure of the sample from equipartition, being equivalent mathematically to Pearson's test of agreement between observation and expectation. The distribution of $\frac{1}{2}\chi^2$ is well represented by a smooth curve independent of $\bar{x}$ of the form (Pearson's Type 3)

$$df = \frac{1}{\frac{n-3}{2}!}\, t^{\frac{n-3}{2}}\, e^{-t}\, dt.$$

and the frequency with which χ^2 exceeds successive integral values, has been tabulated by Elderton (4, 1902 and 5, 1914) for values of n from 0 to 30.

We are therefore in a position to test whether the conditions which lead to the Poisson Series are in fact fulfilled in any given body of bacterial data for which the counts on individual plates are known; it is only necessary to calculate the above index of dispersion (χ^2) from each set of parallel plates, and to determine whether the distribution of this index is or is not in accordance with the distribution predicted from Elderton's tables, when

$$\chi^2 = \frac{1}{\bar{x}} S\,(x - \bar{x})^2$$

and $$n' = n.$$

The statistic χ^2 thus supplies an index of dispersion for sets of parallel plates. If the bacterial counts conform to the Poisson distribution the average value of χ^2 will be one less than the number of plates. For sufficiently numerous sets of plates the agreement may be tested more exactly by the use of Elderton's Tables.

6. The χ^2 Index of Dispersion applied to Cutler's Data

The values of χ^2 obtained from the sets of four parallel plates, grouped according to the value of the mean, are shown in Table VI.

Table VI

χ^2

$\bar{x}$	·5	1·5	2·5	3·5	4·5	5·5	6·5	7·5	8·5	9·5	10·5	> 11	Total
20	2	—	—	—	—	—	—	—	—	—	—	—	2
30	1	—	—	—	—	—	—	—	—	—	—	—	1
40	2	—	2	—	2	1	—	—	—	—	—	—	7
50	—	1	2	5	2	1	1		—	—	—	—	12
60	5	3	2	—	—	—	—	2	—	—	—	12·3	13
70	3	2	4	—	1	—	1	—	—	—	—	13·6, 16·9	13
80	1	1·5	2·5	1	1	2	—	—	—	—	—	—	9
90	2	3	1	3	1	—	—	—	—	1	—	—	11
100	1	3	3·5	—	—	—	—	1	1	—	—	14·8, 24·5	11·5
110	—	2·5	1	1	—	—	—	—	—	—	—	—	4·5
120	2	—	—	1	—	—	1	—	—	—	—	15·1	5
130	—	—	1	·5	—	1	—	—	—	—	1	14·2	4·5
140	—	—	2	1·5	1	1	—	—	—	—	—	—	5·5
150	3	1	5	1	—	—	—	—	—	—	—	—	10
160	6	1	—	—	—	—	—	—	—	—	—	—	7
170	1	—	—	1	—	—	—	—	1	—	—	17·5	4
180	4	1	1	—	—	—	—	—	1	—	1	24·0, 13·9	10
190	1	—	·5	—	—	—	—	—	—	—	—	—	1·5
200	2	1	·5	—	—	—	—	—	—	1	—	15·8	5·5
210	1	1	—	1	—		—	—	—	—	—	—	3
220	—	—	1	—	—	—	—	1	—	—	—	12·2, 16·8	4
230	—	—	—	—	1	—	1	—	—	—	—	21·4	3
240	—	—	1	—	2	1	—	—	—	—	—	—	4
250	1	—	—	—	—	—	—	—	—	—	—	—	1
260	1	—	—	—	—	—	—	—	—	—	—	11·4	2
270	—	1	—	—	—	—	—	—	—	—	—	29·1	2
	39	22	30	16	11	6	5	4	3	2	2	16	156

No obvious relationships are observable between the value of χ^2 and that of $\bar{x}$. There is indeed an excess of the exceptionally large values of

χ^2 (> 11) among the higher values of $\bar{x}$, but this on investigation proved to be completely accounted for by the epidemic character of the occurrences of these large values, which we shall demonstrate below (see Fig. 2). The longest and most severe epidemic occurred during a period (Oct.–Dec.) when the bacterial numbers were generally high. Within this period no sensible association is apparent.

Confining attention therefore to the distribution of χ^2, irrespective of the mean number of colonies counted, it is clear that the sets with exceptionally large variations, which interfered with the preliminary reduction of the data, are now distinguishable as those with high values of χ^2. If the sets were random samples of Poisson Series, it appears from Elderton's Tables that only 3 per cent. of the observed values should exceed 9. It is clear that there is here a group which must be excluded in considering the agreement of the remainder with the theoretical distribution. If this were the only irregularity in the observed numbers we should therefore compare them with a theoretical series having the same total below 9. As it is there is also some irregularity visible at the beginning of the series, suggesting that there is also an excess of unduly small values of χ^2. For this reason we shall base our comparison on the total observed between 1 and 9, as is shown in Table VII.

Table VII

Comparison of observed and expected distribution of χ^2, *4-plate data.*

χ^2	Expected m	Observed $m + x$	Difference x	$\frac{x^2}{m}$
·5	24·97	39	+ 14·03	
1·5	28·76	22	− 6·76	1·589
2·5	22·72	30	+ 7·28	2·333
3·5	16·36	16	− ·36	·008
4·5	11·27	11	− ·27	·006 } ·136
5·5	7·56	6	− 1·56	·322
6·5	4·99	5	+ ·01	·000
7·5	3·25	4	+ ·75	·173 } ·266
8·5	2·10	3	+ ·90	·386
over 9	3·68	20	$\chi^2 = 4·817, 4·324$	
Total	125·66	156	$P =$ ·682, ·232	

Within the range from 1 to 9, the agreement of the observed with the expected values is striking. When tested in eight groups, the probability of obtaining a worse fit by chance from perfectly normal data is ·682, and even when grouped in the most unfavourable manner, by throwing together consecutive positive and negative residuals, a method suggested by Mr Udny Yule, the probability is still ·232. There is therefore no significant deviation of those values from expectation.

Of those above 9, we may anticipate that some three or four will be normal values and the remainder exceptions. It is of course impossible to separate these with absolute certainty. In discussing the evidence for epidemics we shall assume that the four values below 11 are normal and that the remainder are exceptions. When, however, the fact of the epidemic incidence of those exceptional values is taken into account, it appears that the two between 10 and 11 are among the relatively few "normal" sets occurring in an epidemic period and are therefore probably exceptions, while the two between 9 and 10, and possibly also the value at 11·4, are for the same reason probably normal.

It is thus possible to separate this class of exceptions from the remaining data with some degree of certainty and to study them individually, but this is not possible for the exceptionally invariable sets. All that we can do here is to show that the evidence for their real existence is stronger than appears in Table VII. If we subdivide the region of the first two groups of that table somewhat more closely we obtain

Table VIII.

χ	Expected	Observed
0		
	11·82	21
·75		
	9·97	12
·95		
	12·56	17
1·15		
	14·15	9
1·35		

the excess of numbers is most clearly marked in the group of smallest values, and is possibly though not certainly confined to the region.

These conclusions are independently confirmed by the sets of five parallel plates. In Table IX is shown a comparison of the observed distribution with that expected, on the basis of the total observed between 2 and 11.

The agreement with expectation in the range from 2 to 11 is perfectly satisfactory; when tested in the 9 unit groups, the possibility of obtaining a worse fit by chance from normal data is ·765. Grouping together the consecutive positive and negative errors, it only falls to ·571. There is again no significant deviation of the distribution in this range from expectation.

Table IX

Comparison of observed and expected distribution of χ^2, *5-plate data*

χ^2	Expected m	Observed $m+x$	Difference x	$\frac{x^2}{m}$	
·5	10·94	25	+14·06		
1·5	21·10	27	+ 5·90		
2·5	21·58	24	+ 2·92	·271	·402
3·5	18·41	20	+ 1·59	·137	
4·5	14·39	12	− 2·39	·397	
5·5	10·69	11	+ ·31	·009	·146
6·5	7·67	9	+ 1·33	·231	
7·5	5·37	5	− ·37	·025	1·826
8·5	3·70	0	− 3·70	3·700	
9·5	2·51	3	+ ·49	·096	·157
10·5	1·68	2	+ ·32	·061	
over 11	3·22	18	χ^2 = 4·927, 2·938		
Total	121·26	156	P = ·765, ·571		

Of the values above 11, three lie between 12 and 13, and in discussing the evidence for epidemics we shall assume that these are normal sets, and that all those above 13 are exceptions. When we take the evidence of epidemic incidence into account, it is found that the only four sets above 13 which might reasonably be considered normal all occur in epidemic periods, and that the same is true of one out of the three between 12 and 13. This therefore (No. 160, see Fig. 2) is probably also an exception.

The conclusions to be drawn from the 4-plate and from the 5-plate data, thus confirm each other at every point. In both groups the sets having exceptionally high variability may be identified in almost every case with certainty. The majority of both groups, about 124 of the 4-plate sets, and about 117 of the 5-plate sets, are evidently true samples of the Poisson Series. Both groups show an excess of cases of small variability, but it is not possible to specify the actual sets affected by this; it is evident that this cause, like that which produces high variability, is sporadic and not systematic in its action; it affects a certain number of sets in a definite manner, leaving the majority unaffected. This effect, whatever be its nature, is more clearly brought out in the

5-plate than in the 4-plate sets, possibly because the sets of five plates make possible a closer scrutiny into the exactitude of the agreement between the observed sets, and samples from a Poisson Series.

For the same reason the 50 sets of three plates cannot be expected to provide much additional information. The seven exceptionally high values stand out perfectly clearly; the lowest is 9·2, a value which would be exceeded by only one normal sample (of 3) in 100. The next highest values 5·4 and 6·4, would not be suspect save for their occurrence in December; they will be treated as normal.

Since the 3-plate sets are relatively scanty, we can best test their agreement with theory by dividing the theoretical distribution of 43 values at its quintiles, so that the expectation is the same in each group. We then have

Table X. Sets of three plates

$\chi^2 = 1{\cdot}77 \qquad P = {\cdot}775$

χ^2	Expected m	Observed $m+x$	x^2
0			
	8·6	8	·36
·4464			
	8·6	6	6·76
1·0216			
	8·6	11	5·76
1·8326			
	8·6	8	·36
3·2190			
	8·6	10	1·96
Total	43	43	15·20

The agreement with expectation is excellent, and the sets of three plates bear out the conclusions derived from the sets of four and five plates, save that here there is no visible excess of low values of χ^2.

It appears therefore that out of the 362 sets of plates examined the majority represent true samples from the Poisson Series, such as would be the case if the biological and technical difficulties of the bacterial count method as applied to soil had been completely surmounted. Forty sets, which can be identified almost with certainty, are affected by some cause or causes which greatly increase the variability between the plates, while probably a smaller number, including apparently none of the 3-plate sets, are affected by a second cause of error, which reduces the variability between the plates.

7. The exceptionally Variable Sets in Cutler's Data

The records of the exceptionally variable sets of plates which occurred in Cutler's data, when identified by the method of the preceeding section, were studied individually with a view to gaining light upon the cause of their occurrence. As it is not necessary to reproduce the whole of the statistical tests which were applied, we shall confine ourselves to the main facts which emerged, and which served to justify the previous conclusions, as well as to indicate the nature of the disturbing cause.

The following facts appear to be unquestionable:—

(1) The proportion of exceptionally variable sets is the same for the sets of three, four and five plates in each portion of the total period.

(2) The proportion of exceptionally variable sets varies greatly at different periods, the exceptions occurring in well marked epidemics.

The evidence for these statements may be put in the form of a triple contingency table (see Fig. 2)

Table XI

	Excessively variable				Not excessively variable				Total				
Period	5	4	3	Total	5	4	3	Total	5	4	3	Total	χ^2
1	1	—	1	2	9	9	9	27	10	9	10	29	·967
2	3	2	1	6	7	12	6	25	10	14	7	31	1·728
3	—	—	1	1	12	18	4	34	12	18	5	35	6·176
4	3	(10) 9	1	(14) 13	5	(11) 12	4	(20) 21	8	21	5	34	·818
5	(6) 5	(5) 4	1	(12) 10	(6) 7	(14) 15	4	(24) 26	12	19	5	36	1·733
6	—	—	—	—	19	18	—	37	19	18	—	37	—
7	—	—	—	—	22	13	1	36	22	13	1	36	—
8	—	—	1	1	20	11	5	36	20	11	6	37	5·310
9	1	—	—	1	17	12	6	35	18	12	6	36	1·029
10	2	1	1	4	23	20	4	47	25	21	5	51	1·299
Total	(16) 15	(18) 16	7	(41) 38	(140) 141	(138) 140	43	(321) 324	156	156	50	362	19·060

in which the whole of the 362 observations are divided,

(1) according to the number of plates observed,

(2) in ten periods of time of alternately 36 and 37 days, into which the year was divided,

(3) according as they are judged to be exceptionally variable, or not, solely upon the evidence of the χ^2 index. The subdivision which would be made taking also into account the evidence for epidemics is shown in brackets, but in discussing the evidence for epidemics these modifications are ignored.

To test the first point, each line of Table XI is treated as a 2 × 3 contingency table, and the value of χ^2 calculated from it. It has been shown (Fisher, 1922 (6)) that as in such a table there are two degrees of freedom, χ^2 will be distributed, if there is no association, as in Elderton's Tables when $n' = 3$. To show that at no period is there significant association, the values of χ^2 for the 10 periods are added, and the resulting quantity should be distributed as in Elderton's Tables when $n' = 21$. Since in two consecutive periods no exceptionally variable sets occurred, these periods have been omitted, and n' is taken to be 17. It will be seen from the table that all the values of χ^2 are less than 2, except in two periods in which only a single exceptionally variable set occurred. Such cases are evidently beyond the range of effective application of the χ^2 test, but even including these high values, $P = \cdot 266$, and therefore there is no significant departure from the rule that sets of three, four and five plates show equal proportions of exceptions in all sections of the period of observations.

This fact confirms the justness of the criterion by which the exceptions have been identified, for any error in the method of identification would naturally show itself in the proportion of cases regarded as exceptions; in the second place it indicates that the cause of exceptional variability is not connected with the causes which lead to the rejection of individual plates (contamination, development of fungi or overgrowth by *B. dendroides*), and in the third place it shows that the exceptions are not caused by the exceptional deviation of a single plate, for in this case the proportion of 5-plate sets would necessarily be highest. The third conclusion is borne out by an examination of the numbers counted on individual plates, and both it and the second conclusion are more decisively drawn from the contingency table by ignoring the period of occurrences.

Table XII

No. of plates	5	4	3	Total
Exceptionally variable ...	16	18	7	41
Not exceptionally variable ...	140	138	43	321
Total	156	156	50	362

The numbers in the smaller groups are here sufficient to make a satisfactory test, and the value of P, $\cdot 739$, shows distinctly that there is

CUTLER'S DATA
SETS OF PLATES FOR EACH DAY OF THE YEAR

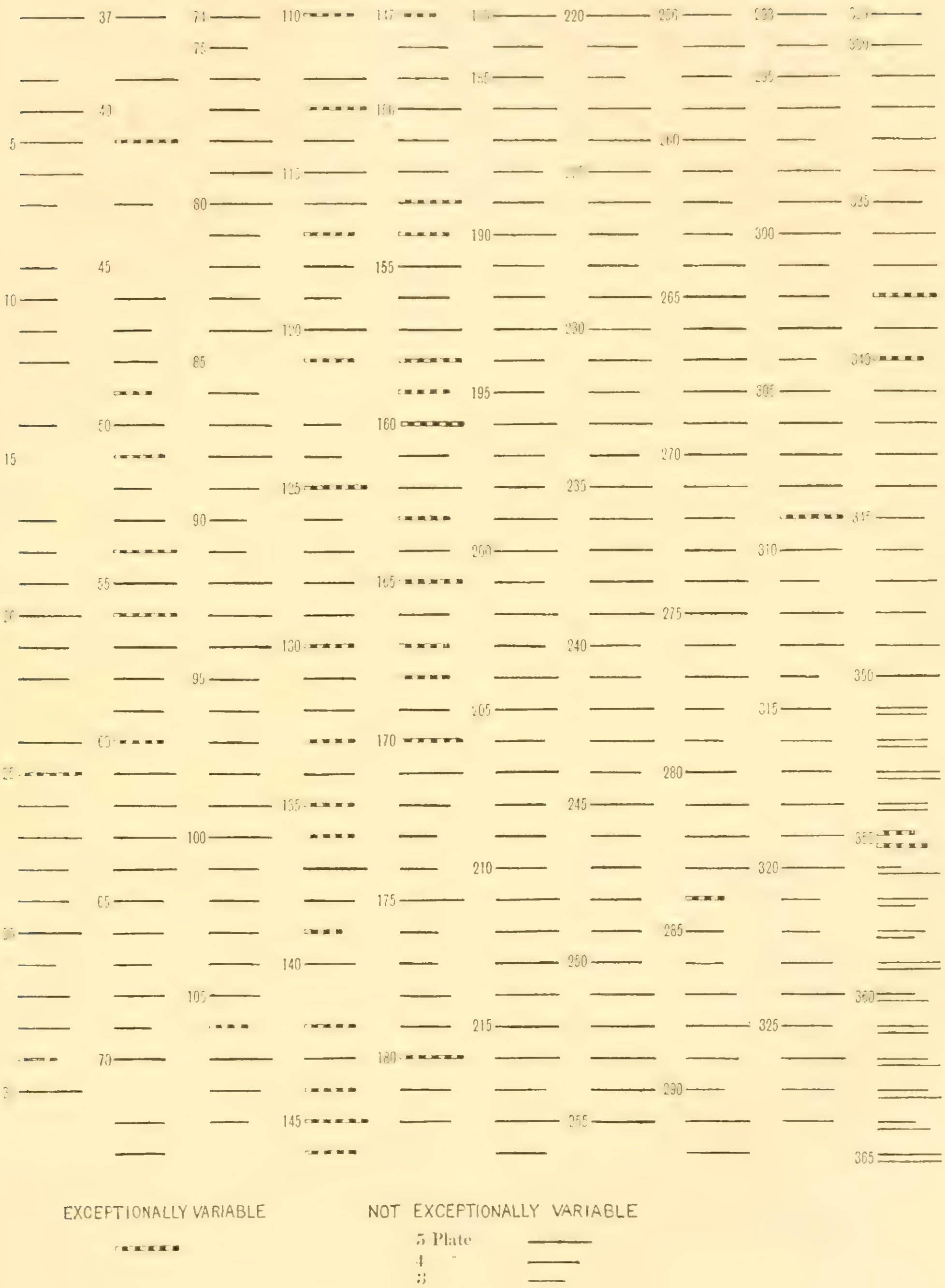


Fig. 2.

no significant difference in the proportion of exceptions between the several groups of observations.

Similarly the distribution of the exceptions in time, in which we have shown the different groups to agree, may be best shown by taking the totals, irrespective of the number of plates in each set. If this is done we have a 2 × 10 contingency table, of which the value of χ^2 proves to be 57·826.

Since $n' = 10$, the chance of such a distribution occurring under conditions of random occurrence in time is about 4×10^{-9}. It is indeed obvious from inspection of Fig. 2 that the exceptional values occur in groups together, although perfectly normal values continue to occur throughout the worst of these epidemics. During the first outbreak seven exceptions occurred with 14 normal values among them; the second epidemic period was more prolonged and included 27 exceptions and 46 normal values. In the second half year of the experiment only six exceptions occurred, of these two occurred on the same day (355) during the last fortnight, when duplicates were taken, and two others, 338 and 340, were but two days apart.

Bearing these points in mind, we have no hesitation in concluding, on purely statistical evidence, that the exceptionally variable sets of platings were due to two causes:—(*a*) a predisposing cause which is at work throughout the epidemic period, and (*b*) some additional circumstance, in the absence of which the counts obtained will still be normal.

8. SPECIAL ORGANISMS WHICH AFFECT THE NUMBER OF COLONIES DEVELOPING

In the daily counts above considered, a uniform technique was followed throughout, and fresh batches of medium were made up at frequent intervals. It is conceivable that occasional differences in plating technique, in the medium, or in counting the plates may by chance have occurred on certain days. It is however most unlikely that any such differences can have extended over the long periods covered by the epidemics of high variance, without the fact being noticed. In seeking a predisposing cause of variance, covering these periods, therefore, one's attention is naturally drawn to possible changes in the soil itself or in its population.

It is known that certain micro-organisms, when growing on the medium, exert an inhibitory action on the development of colonies by other forms. The appearance of such an organism in the soil population, during certain periods, might therefore give rise to periods of higher variation between parallel plates, for unless present in very large numbers it would not appear on all the plates or even in every batch of five plates.

An example of high variation between parallel plates, that was actually traced to such an organism, is given to illustrate this cause of inaccuracy.

The soil used in this case was from the Leeds Experimental Farm, and had received a treatment of naphthalene. Thirty parallel platings of this soil were made on Thornton's agar. The counts of colonies on these plates are given in Table XIII.

Table XIII

Parallel plates of Leeds soil

Plate No.	Number of colonies	Plate No.	Number of colonies
1	*240*	16	126
2	*209*	17	126
3	*177*	18	126
4	*158*	19	121
5	157	20	120
6	*154*	21	119
7	151	22	118
8	137	23	117
9	136	24	114
10	132	25	113
11	131	26	109
12	131	27	99
13	130	28	*91*
14	128	29	*91*
15	127	30	*87*

χ^2 Index. Whole series = 230·17
Minus the italicised plates = 27·81

It will be seen that the variation between parallel plates in the whole series is excessive. In examining the plates, some were found to contain an organism forming a growth between the agar and the bottom of the dish. This organism occurred on the plates italicised in Table XIII. It is a motile organism and apparently spreads in the water film underlying the agar. On plates 28, 29 and 30, the growth of this organism was sheet-like and from the low counts obtained it would appear that its growth has reduced colony development. On plates 1, 2, 3, 4 and 6, it has produced a number of separate colonies underlying the agar. These colonies were probably produced by individuals which had multiplied and migrated along the bottom of the dish after the agar had set, but could not be separated from other colonies in counting the plate. The counts on these plates are therefore excessive. The presence of this organism on the bottom of the plates has thus produced an abnormal variation in the whole series. It will be seen that, if plates on which it occurs are ignored, the χ^2 index for the remaining 22 plates falls within the expectation of random sampling.

A pure culture of this organism was obtained and a plating from a sample of Rothamsted soil was made, a small loopful of suspension of the organism being added to the first dilution flask. Table XIV, Series A, shows the colonies developing on six parallel plates of the soil thus treated, compared with a control series of plates of the same soil not inoculated, Series B, which were made at the same time.

Table XIV

Effect of Leeds soil organism on colony development from suspension of Rothamsted soil

Series A. Suspension inoculated			Series B. Control	
Plate No.	Number of colonies	Area of bottom spreading	Plate No.	Number of colonies
1	85	nil	1	95
2	79	nil	2	90
3	78	nil	3	86
4	70	nil	4	85
5	58	nil	5	85
6	60	2·25 sq. cms.	6	82
7	56	7·75 ,,	7	81
8	45	27·0 ,,	8	77
9	41	54·5 ,,	9	73
10	39	56·5 ,,		
χ^2 Index, Plates 1 to 5 = 5·86 Plates 1 to 10 = 40·01			χ^2 Index = 1·89	

In this case the organism formed a spreading growth over the bottom. The area of this spreading growth, where it occurred, was measured and is shown in Table XIV. It will be seen that the reduction in colony development is clearly related to the amount of spreading growth. In this series of plates it is also evident that the variation is greatly increased by the occurrence of the organism on certain of the plates.

From an abnormally variable series of plates of Rothamsted soil a second organism has been isolated, whose frequent habit it is to spread on the under surface of the agar, and which has a similar inhibitory action on the development of other colonies. Table XV shows two sets of plates of a suspension of Rothamsted soil, one set of which was inoculated with this organism. The reduction of, and increased variation in colony numbers are again well seen.

Table XV

Effect of toxic organism from Rothamsted soil on colony development from a soil suspension

Series A Plates inoculated		Series B Control	
Plate No.	Number of colonies	Plate No.	Number of colonies
1	192	1	179
2	168	2	171
3	147	3	168
4	130	4	150
5	127	5	150
6	113		
Mean 146·1 χ^2 Index = 29·47		Mean 163·6 χ^2 Index = 4·17	

It is of course impossible to decide, with certainty, from a simple record of colony numbers, whether the presence in the soil of some such organism was the cause of the epidemics of variable plate-sets in Cutler's series. However, the above two cases of high variance between parallel plates, which have been traced to the presence of definite organisms, show that this factor, though apparently of infrequent occurrence, is capable of causing a disturbance in the colony numbers of precisely the kind actually observed. It is important to notice that this, probably like all other causes, that produce a sensible departure from the Poisson Series, seriously disturbs the mean value.

9. THE OCCURRENCE OF SUBNORMAL VARIATION

It has been shown that in a small proportion (about 34 cases) of Cutler's data, the variation between parallel plates has been apparently lowered by some disturbing agency. The same phenomenon in a much aggravated form appears in Owen's data (section 10), and has from time to time occurred in Thornton's work. For example the 20 plates shown in Table I display an unduly low variation, and though this fact does not detract from the value of the data in proving the equivalence of parallel dilutions, it does throw suspicion on the value of the mean as an estimate of bacterial density. A similar depression appears in Table XIV, Series B.

Unlike the excessively variable sets, the sets with subnormal variance cannot be identified individually in Cutler's data, and we have therefore less evidence upon which to put forward a biological explanation of the phenomenon; certain facts, however, concerning observations made in the course of 1921, suggest that additional precautions in the preparation of the medium, may be effective in eliminating the disturbing cause.

The additional data were accumulated in the Bacteriological Department[1] in the summer and autumn of 1921 in the course of some work on the relationship of bacterial numbers to nitrate content in the field soil. In each of these experiments a series of some 45 samples of soil were taken from a plot 9 by 15 feet in area and the bacterial numbers in each sample estimated by the plate method using Thornton's agar medium. The first experiment was carried out with the dunged plot in Barnfield. The technique used was similar to that employed in Cutler's work, five parallel platings being made of each sample and the colonies counted after an incubation of seven days at 20° C.

Of the 33 sets available, three show excessive variance, the remainder are distributed as in Table XVI.

Table XVI

$\chi^2 = 3{\cdot}08$ $P = {\cdot}381$

χ^2	5-plate	4-plate	3-plate	Total	Expected	x^2/m
·5	—	—	1	1	9·78	·79
1·5	4	2	—	6		
2·5	2	—	—	2	9·39	·21
3·5	4	2	—	6		
4·5	4	—	1	5	5·56	·37
5·5	—	2	—	2		
6·5	3	1	—	4	5·06	1·71
7·5	3	—	—	3		
8·5	1	—	—	1		
Total	21	7	2	30		3·08

It will be seen that these agree well with the Poisson Series, and show no sign of subnormal variation.

A second experiment was carried out at Kingsthorpe Hall, Northampton. The soil is here of a markedly different type from the heavy Rothamsted soil, being a light ferruginous loam. In this experiment the technique was varied in that the colonies on each plate were counted twice, after seven and twelve days' incubation. It will be sufficient to compare the observed and expected values of the total, $S(\chi^2)$, for different groups of plates.

---

[1] The authors wish to acknowledge their indebtedness for the assistance rendered by other Departments at Rothamsted in this work.

Table XVII

Number of plates per set	Medium	After 7 days		After 12 days	
		Expected	Observed	Expected	Observed
4	A	18	13·85	24	27·31
5	A	152	109·33	144	133·96
9	A	8	1·96	8	8·73
Total	A	178	125·14	176	170·00
20	B	19	19·45	19	25·34

In all these groups where medium A is used the variance is distinctly subnormal after 7 days, but is apparently normal after 12 days. With medium B, the variance is normal at both counts. Now the sets of 9 and of 20 plates were parallel dilutions of the same sample, and the mean count from medium A was only 75 per cent. of that obtained on medium B. The abnormality of medium A was afterwards traced to the temperature at which it was filtered, a technical detail which has an important bearing on the ability of the medium to support bacterial growth (Thornton, 1922(11)).

In the comparison given by Thornton(11) of the two batches of medium, identical save that one was filtered at 50° C. and the other at 100° C., 10 plates being prepared from each, the former gave a mean count 79 per cent. of the latter; in this case also the defective medium showed subnormal variance giving a value $\chi^2 = 3{\cdot}2$ (after eight days), whereas the normal medium gave a value 10·3. The former would only occur once in 22 trials by chance, and therefore represents clearly a subnormal condition.

Whatever the biological explanation of subnormal variance may be, it is therefore sometimes indicative of a serious error in the value of the mean. In this respect it is a danger signal which cannot be disregarded. When a set of plates shows excessive variability no one will be tempted to lay too much stress upon their mean; it is obvious in such cases that there is a large probable error, and it has been seen (Section 8), that there will usually be also a considerable systematic error in such cases. A set of plates with abnormally low variance on the other hand, may appear to be particularly good data, although, as we have just seen, this type of abnormality is also indicative of large systematic errors. It is therefore of practical importance that such departures from the Poisson distribution should be detected, whenever they occur. Since subnormal variation cannot be detected with certainty in a small set of plates, we recommend that occasional sets of 10 or 20 plates should be prepared from time to time, and that if necessary every batch of medium prepared should be tested in this way, the colonies being counted after seven days.

10. THE χ^2 INDEX OF VARIABILITY APPLIED TO OTHER BACTERIAL COUNT DATA

It has been shown by the use of the χ^2 index of variability, that the great bulk of Cutler's data on soil bacteria appears to be true samples from the Poisson Series, and that therefore the accuracy of these results is known with precision; also that, by the same method, a small proportion of exceptions may be detected in which some definite disturbing cause has interfered with the accuracy of the results. It is therefore desirable to apply the same test to other sufficiently extensive bodies of material, in order to ascertain if, by other methods, a similar degree of accuracy can be obtained, and failing that, if further light can be thrown on the problems of the dilution method. Data from four sources have been examined in this way.

(A) Buddin's counts of soil bacteria at Rothamsted, using a gelatine medium.

(B) Counts of soil bacteria published by Engberding (1909(12)).

(C) Breed and Stocking's tests of the accuracy of counting *B. coli* in milk (1920(13)).

(D) W. Owen's bacterial counts in sugar refinery products (1914(14)).

In the aggregate we have tested over 1000 sets of parallel plates; owing to the bulk of the total examined it is possible that a small proportion of arithmetical errors has been included, although the application of the method is much more expeditious than that of the preliminary investigation of Cutler's data. Only the obvious and unquestionable features of each body of data will be dealt with.

(A) *Buddin's data*

A very large number of bacterial counts were made at Rothamsted by W. Buddin, to whom we are indebted for permission to make use of these data. The actual plate counts, though not published, formed the basis of bacterial number estimations used in Buddin's work on the effect of antiseptics on soil(15).

The platings in this work were made on a nutrient gelatine having the following composition:—Witte's peptone 40 grams, Lemco 20 grams, NaCl 20 grams, gelatine 480 grams, distilled water 4000 c.c.

The counts therefore supply an example of the degree of accuracy obtained with a gelatine medium, where a considerable source of variance is produced by the occurrence of liquefying organisms on the plates.

From the mass of data available, 100 sets of triplicate platings were extracted. The expected and observed values of χ^2 in this series are shown in Table XVIII.

Table XVIII

χ^2	Expected	Observed	Difference
·5	39·3	25·5	− 13·8
1·5	23·9	26	+ 2·1
2·5	14·5	12	− 2·5
3·5	8·8	10·5	+ 1·7
4·5	5·3	6	+ ·7
5·5	3·2	4	+ ·8
6·5	2·0	3	+ 1·0
7·5	1·2	4	+ 2·8
over 8	1·8	9	+ 7·2
Mean	2·0	3·04	

There is a marked deficiency below 1, and an increasing excess above 3. No distinct class of exceptionally high values can be detected, only three values exceed 10, and none exceed 15. The causes of additional variability probably affect all observations in some degree, and are therefore systematic rather than sporadic. The mean variance is about 50 per cent. in excess of that due to random sampling. As in Cutler's 3-plate data the departure from expectation is best shown by dividing the distribution at the quintiles as in Table XIX.

Table XIX

$\chi^2 = 17{\cdot}4$ $P = {\cdot}0017$

χ^2	Expected m	Observed $m + x$	x^2
0			
	20	12	64
·4464			
	20	15	25
1·0126			
	20	23	9
1·8326			
	20	15	25
3·2190			
	20	35	225
Total	100	100	348

Such a departure from expectation would occur by chance but once in 600 tests; it is therefore clearly significant. The technique used here did not therefore give results of such accuracy that the variance between parallel plates could approximate to the Poisson Series.

(B) *The data of Engberding* (12)

The parallel platings given by this author were made to test various points connected with the plate method of counting soil bacteria. Some of the sets of platings were made on a variety of gelatine and agar media, as a test of these. The majority, however, were poured on an agar medium, containing "Nahrstoff-Heyden," that was considered by the author to be the best of the media tested.

Engberding gives 24 sets of plates; of these, 14 are of six plates each, six of five plates, three of four plates and one of nine plates. Nearly all the sets show excessive variability; only three values out of the 24 are below the expected average for the corresponding number of plates. The total of the 24 values is 5·36 times the expected total. No further test is necessary; random sampling must be regarded as one of the smaller causes of variation in these data.

(C) *The data of Breed and Stocking* (13)

We next come to a very thorough attempt made by Breed and Stocking to test and improve the methods used in the bacterial analysis of milk. The medium used in the platings here considered had the following composition:—"Difco" peptone 1 per cent., lactose 1 per cent., "Lemco" ·3 per cent., air dried agar 1·5 per cent. A single batch of medium was used throughout each experiment, so that ability to reproduce the medium, is not here tested. Parallel samples of normal milk, and of milk inoculated with *B. coli*, were analysed by different analysts and at different stations. Two series of these records have been examined by comparing the different plates of each separate analysis. Each series yielded 132 sets of three numbers, the duplicate counts of the same set of plates being reckoned as two. If the duplicate counts had closely agreed, this would tend to give us a bad fit between observation and expectation, to the extent of doubling χ^2. Though the agreement is not sufficiently great to have this effect, the tendency is to be borne in mind.

The expected and observed distributions are shown in Table XX.

As with Buddin's data, though to a less extent, there is a small systematic excess of the larger values; the mean variance in series B is about 30 per cent. in excess of expectation, while in series C it is only about 20 per cent. Series B also shows certain other irregularities and possibly the occurrence of sporadic causes of variation. Series C, which represents the final perfection of the technique employed, shows no excessively variable sets of plates.

Table XX

χ^2	Expected	Series "B"	Series "C"
·5	51·9	46	43
1·5	31·5	35·5	30
2·5	19·1	14	24
3·5	11·6	6·5	12
4·5	7·0	10	10
5·5	4·3	3	4
6·5	2·6	5	4
7·5	1·6	2	2
over 8	2·4	8	5
Mean	2·00	2·65	2·45

It is, we believe, possible to indicate the cause of the small systematic excess of variance in this exceptionally fine body of data. As has been observed, the duplicate counts, which are recorded in full, do not agree very closely, and it is possible that what may be called "error of counting" is responsible for the existing discrepancy. If we consider such a typical pair of duplicate counts such as that shown in Table XXI, we may regard the mean difference, as due to the personal equation of the analyst; and the departures from the mean as made up of the several "errors of counting" of the set. If the standard "error of counting" is σ, then the mean value of the sum of the squares of the three departures will be $4\sigma^2$. In this way the standard "error of counting" was estimated for each of the main groups of observations in Series C, divided according to the mean number of colonies per plate, and the additional variance ascribable to "errors of counting" expressed as a percentage of the expected variance.

Table XXI

Plate	First count	Second count	Difference	Departure from mean
1	70	68	+ 2	+ 8
2	61	72	− 11	− 5
3	54	63	− 9	− 3
Mean			− 6	

Table XXII

Percentage variance due to "errors of counting"

Colonies per plate about	...	36	62	82	161	364	All
Increased variance per cent.	...	16 %	24 %	13 %	17 %	59 %	22 %

The effect is thus seen to be a fairly uniform one, though distinctly more prominent among the more crowded plates, of which eight pairs of triplets were available. The higher value in the second group is perhaps due to the fact that these contain the counts of the mixed bacterial population in normal milk, while the others are counts of a practically pure culture of *B. coli*.

The effect ascribable to "errors of counting" is thus of just the right magnitude to explain the additional variance observed in Series C. Since all the groups are affected similarly and nearly to an equal extent, we may anticipate that if this explanation is correct, the actual values of Series C will fit the theoretical expectation if a uniform allowance of 20 per cent. is made for the additional cause of variation. The distributions are so compared in equal intervals of χ^2 in Table XXIII, and by sextiles in Table XXIV.

Table XXIII

χ^2	Expectation with 20 % allowance	Observed
·6	51·9	47·5
1·8	31·5	35·5
3·0	19·1	21
4·2	11·6	12
5·4	7·0	5
6·6	4·3	5
7·8	2·6	3
9·0	1·6	—
over 9	2·4	3

Table XXIV

$\chi^2 = 7{\cdot}545$, $P = {\cdot}185$ ($P = {\cdot}584$)

χ^2	Expectation m with 20 % allowance	Observed $m + x$	x^2
0			
	22	14	64
·4378			
	22	28	36
·9732			
	22	24	4
1·6634			
	22	21	1
2·6366			
	22	28	36
4·3003			
	22	17	25
Total	132	132	166

The distribution shown in Table XXIII shows a remarkably close agreement with expectation. A more exact test of agreement is afforded by the division at the sextiles (Table XXIV); the actual figures show but a moderately good fit with $\chi^2 = 7{\cdot}545$, and $P = {\cdot}185$; since however duplicate counts of the same plates have been taken as independent observations, χ^2 has been increased by this cause to some extent short of doubling, so that we may say that in reality χ^2 lies between 3·77 and 7·54, while P lies between ·584 and ·185; neither value could be taken as indicating a significant departure from expectation.

We believe, therefore, that in this material, at all events in Series C, the somewhat severe conditions under which the Poisson Series is produced, were in reality fulfilled, and that the departure of the observations from expectation could have been eliminated had precautions been taken to secure a sufficiently accurate counting of the colonies. It must however be borne in mind that the material employed consisted in nearly all cases of almost pure cultures of *B. coli* in milk. The case cannot therefore be compared closely to the different problem of counting such a mixed bacterial flora as occurs in soil, where many different types of organisms, whose growth may be mutually harmful, occur on the plates.

The interference on the plates between dissimilar organisms cannot here be seen, neither can the capability of the medium to check this interference be studied. In this material, for example, there would be little danger of frequent interference by "spreading" organisms, whose growth, had they occurred, would probably have been stimulated by such a medium as was used, containing peptone and meat extract.

The lessened accuracy in counting a mixed flora on this medium is illustrated in Table XXII, where the second group of platings, which contains counts of uninoculated milk, shows a noticeably higher variance in counting than the adjoining groups made from milk cultures of *B. coli*.

The data show, however, that when such a simplified flora is studied, an agreement between parallel platings comparable with the expectations of random sampling can be obtained.

(D) *The data of W. Owen* (14)

One of the most remarkable bodies of data which we have examined is that provided by W. Owen in his investigation of various culture media for the counting of micro-organisms in cane sugar products. In this work, a variety of different media were employed, varying in composition, reaction and osmotic pressure. These were tested in counting bacteria from a variety of sugar refinery products. From the variety of media employed, and from the fact that most of them were new and of untested value, it was to be expected that a rather high variance between parallel platings would be found over the whole series taken together. Had this been the case, separate tests would have been needed of the indices of variance on the separate media. In fact, however, no such remarkably high variance was found.

The analyses were performed with sets of six plates, and we have chosen the first 100 of these sets for examination. The expected and observed numbers are shown in Table XXV.

Table XXV

χ^2	Expected	Observed	Expected 43%
·5	3·7	38	1·6
1·5	11·3	15	4·9
2·5	14·9	6	6·5
3·5	15·0	9·5	6·5
4·5	13·4	6	5·8
5·5	11·0	3·5	4·8
6·5	8·5	3	3·7
7·5	6·4	3	2·8
8·5	4·7	1	3·5
9·5	3·4	1	
10·5	2·4	—	3·4
11·5	1·7	1	
12·5	1·1	2	
13·5	·8	—	
14·5	1·6	—	
15·5		1	
over 16		10	

The excess of highly variable sets occasions no surprise; we have met with this feature in about the same proportion in Cutler's data. What is astonishing in this case is the immense excess of sets less variable, and in the majority of cases much less variable, than would be the case under undisturbed conditions of random sampling.

In the fourth column we have shown the expected distribution fitted to the total number in the range from 2 to 14. This seems to agree with the distribution observed within this range. We are unwilling to lay much stress on this explanation since the agreement is based on only 36 observations. If it were accepted it would imply that the conditions which lead to the Poisson Series were really operative in about 44 per cent. of the cases, that in at least 10 and probably 11 per cent. excessive variability has been produced, and in the remaining 45 per cent. the variability has been abnormally depressed.

The extent to which the differences between the counts of parallel plates is diminished seems to put the phenomenon beyond the reach of the ordinary explanations; there are some indications, for example, that the plates have not been in all cases completely counted, but it is difficult to imagine that this cause could be responsible for any such bias as is observed, in view of the fact that a probable error is calculated separately from each set. Severe competition between colonies on the plate is admittedly a possible cause of diminished variability, but we cannot imagine it acting with such severity as would be necessary to explain these results, especially as in the 38 cases in which χ^2 is less than one, the mean number of colonies per plate is always less than 100, and in 15 cases is less than 10.

In more than one instance all the six plates have an equal number of colonies; in samples from a Poisson Series, this would occur but very rarely. For 13 colonies on each plate for example, as is recorded in one instance, the most favourable assumptions will only allow such a coincidence once in some 25,000 trials. Since in the majority of these counts we clearly are not dealing with undisturbed conditions of random sampling, the point cannot be pressed further. We do not agree, however, with the statement that, when such a coincidence occurs, the probable error is zero.

In reviewing the foregoing data, it seems probable that the action of liquefying bacteria, and the development of rapidly growing organisms, unchecked by the medium employed, were the main causes of excessive variance between parallel platings in the work of Buddin and Engberding respectively.

It appears, however, that the conditions of accuracy, such that the development of colonies on parallel platings will form a Poisson Series, can be fulfilled in dealing with a simplified bacterial flora (Breed and Stocking), and have been approached in dealing with the mixed micro-flora of soil, where the medium used has been so devised as to check the excessive development of spreading organisms, as in the case of Thornton's medium. It is possible that these conditions of accuracy would be fulfilled with greater certainty in the case of a mixed micro-flora, if the medium could be further improved so that it checked the growth of such harmful organisms as that found in the Leeds soil (p. 345).

CONCLUSIONS

(1) Under ideal conditions the bacterial counts on parallel plates will vary in the same manner as samples from a Poisson Series. When these conditions are fulfilled the mean count of a number of plates is a direct measure of the density of the bacterial population considered (though not, of course, of the total bacterial flora); and the accuracy of such an estimate is known with precision.

(2) For any considerable body of records of sets of parallel plates, agreement with this theoretical distribution may be tested by means of the index of dispersion

$$\chi^2 = \frac{1}{\bar{x}} S (x - \bar{x})^2,$$

where $\bar{x}$ is the mean, and x any individual number of colonies counted on a plate (see Section 5).

(3) From an examination of several large bodies of data we conclude that accurate conformity with the theoretical distribution, though rare, is not unattainable. In particular with a carefully improved technique, and a relatively simple bacterial flora, we believe that the conditions have probably been fulfilled by Breed and Stocking; secondly, by the aid of a specially adapted medium Cutler and Thornton have shown that these conditions have been accurately reproduced, in the great majority of cases, even with the mixed bacterial flora of the soil.

(4) *Any significant departure from the theoretical distribution is a sign that the mean may be wholly unreliable.*

(5) Excessive variance may be produced by the occurrence of certain soil organisms, which have been isolated, and which exert a toxic influence on other forms, and in one case disturb the counts by multiple colony formation.

(6) Subnormal variance is in our experience indicative of some defect in the composition of the medium.

REFERENCES

(1) Poisson (1837). *Recherches sur la probabilité des Jugements.* Paris.

(2) "Student" (1907). On the error of counting with haemocytometer. *Biometrika,* v. 351.

(3) Fisher, R. A. (1921). On the mathematical foundations of theoretical statistics. *Phil. Trans.* A. ccxxii. 309.

(4) Elderton, P. (1902). Tables for testing goodness of fit. *Biometrika,* i. 155.

(5) Pearson, K. (1914). *Tables for statisticians and biometricians.* Camb. Univ. Press.

(6) Fisher, R. A. (1922). On the interpretation of χ^2 from contingency tables, and the calculation of *P. J.R.S.S.* lxxxv. 87.

(7) Soper, H. E. (1914). Tables of Poisson's exponential binomial limit. *Biometrika,* x. 25.

(8) Whitaker, L. (1914). On the Poisson law of small numbers. *Biometrika,* x. 36.

(9) Bateman, H. (1910). Note on the probability distribution of α particles. *Phil. Mag.* Series vi. vol. xx. p. 704.

(10) Bortkiewicz, L. von (1898). *Das Gesetz der kleinen Zahlen.* Leipzig.

(11) THORNTON, H. G. (1922). On the development of a standardised agar medium for counting soil bacteria, with especial regard to the repression of spreading colonies. *Annals of Applied Biology*, IX.

(12) ENGBERDING, D. (1909). Vergleichende Untersuchungen über die Bakterienzahl im Ackerboden in ihrer Abhängigkeit von ausseren Einflussen. *Centralblatt für Bakteriologie*, Abt. II. Bd. 23, p. 569.

(13) BREED, R. S. and STOCKING, W. A. (1920). The accuracy of bacterial counts from milk samples. New York Agr. Exp. Station. *Technical Bulletin*, No. 75.

(14) OWEN, W. (1914). Investigation of the comparative values of various culture media for the quantitative determination of microorganisms in cane sugar products. *Centralblatt für Bakteriologie*, Abt. II. Bd. 42, p. 335.

(15) BUDDIN, W. (1914). Partial sterilisation of soils by volatile and non-volatile antiseptics. *Journal of Agricultural Science*, VI. 417.

(16) WYANT, Z. N. (1921). A comparison of the technic recommended by various authors for quantitative bacteriological analysis of soil. *Soil Science*, XI. 295.

(17) CUTLER, D. W., CRUMP, L. M., and SANDON, H. (1922). A quantitative investigation of the bacterial and protozoan population of the soil, with an account of the protozoan fauna. *Phil. Trans. Roy. Soc.* B.

(*Received July* 21*st*, 1922.)

CAMBRIDGE : PRINTED BY
J. B. PEACE, M.A.,
AT THE UNIVERSITY PRESS

Vol. IX, No. 1 APRIL, 1922

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

PUBLICATIONS COMMITTEE

V. H. BLACKMAN
A. W. HILL
G. A. K. MARSHALL
A. D. IMMS
R. T. LEIPER

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, Manager
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 28, ESSEX STREET, LONDON, W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES AND CANADA)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

The Association of Economic Biologists

President

Prof. E. B. POULTON, M.A., D.Sc., LL.D., F.R.S.

Vice-Presidents

Prof. V. H. BLACKMAN, Sc.D., F.R.S.
G. A. K. MARSHALL, C.M.G., D.Sc.

Hon. Treasurer

A. D. IMMS, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Editors

W. B. BRIERLEY, D.Sc.
D. WARD CUTLER, M.A.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (General and Botanical)

W. B. BRIERLEY, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (Zoology)

J. WATERSTON, M.A., D.Sc.,
Nat. Hist. Museum,
South Kensington, S.W. 7

Council

A. W. BACOT, Esq.
W. LAWRENCE BALLS, Sc.D.
W. F. BEWLEY, D.Sc.
Prof. V. H. BLACKMAN, Sc.D., F.R.S.
F. T. BROOKS, M.A.
A. B. BRUCE, M.A.
E. J. BUTLER, D.Sc., C.I.E., M.B.
G. A. K. MARSHALL, C.M.G., D.Sc.
J. W. MUNRO, D.Sc.
S. A. NEAVE, D.Sc.
Prof. J. PERCIVAL, M.A.
E. J. RUSSELL, D.Sc., F.R.S.

CONTENTS OF Vol. IX, No. 1

PAGE

1. The Control of the Greenhouse White Fly (*Asterochiton vaporariorum*) with Notes on its Biology. By Ll. Lloyd, B.Sc. (Leeds). (With 5 Text-figures, 2 Diagrams and Plates I and II) 1
2. Observations on the Ensheathed Larvae of some Parasitic Nematodes. By T. Goodey, D.Sc. (With 1 Text-figure) 33
3. Leaf Character in Reverted Black Currants. By A. H. Lees, M.A. (With 46 Text-figures and 11 Graphs) 49
4. Further Observations on *Sitones lineatus* L. By Dorothy J. Jackson, F.E.S. (With 2 Text-figures) 69
5. Contributions to the Biology of Freshwater Fishes. I. The Effects of various Impurities in a Stream on the Life of Spermatozoa of Trout and Young Trout. II. Biological Problems connected with a Trout Farm. By W. Rushton, A.R.C.S., D.I.C., F.L.S. . . . 72
6. Obituary Notice. Dr Caroline Burling Thompson, 1869–1921 . 81
7. List of Members of the Association of Economic Biologists for 1921–2 83
8. Laws of the Association of Economic Biologists 90

Cambridge University Press

Insect Pests and Fungus Diseases of Fruit and Hops. A Complete Manual for Growers. By P. J. FRYER, F.I.C., F.C.S. Crown 8vo. With 24 plates in natural colours and 305 original photographs and diagrams. 45s net.

The present volume represents a careful and painstaking attempt to produce as complete a book of reference as possible, suited to the requirements of the fruit and hop grower, and presented in such a form that the information, while given with scientific precision, is also in a readily available form.

"In modern commercial fruit culture results depend very largely on the grower's ability to control the insect pests and fungous diseases which attack his trees. To assist him in this work he has long needed a reliable handbook covering the whole subject, enabling him to identify his enemies without loss of time, and indicating the best and up-to-date methods of control. Such a book it has been the object of the author of this volume to supply, and it may be said at once that he has succeeded admirably.... He has a particularly clear and orderly style of presenting his facts, giving all the information necessary to the practical man without forcing him to sift out unessential details.... Altogether the book deserves to become the standard reference work for growers."—*The Gardeners' Chronicle*

Cattle and the Future of Beef-Production in England. By K. J. J. MACKENZIE, M.A. With a Preface and Chapter by F. H. A. MARSHALL, Sc.D. Demy 8vo. 7s 6d net.

"One of the best treatises issued in recent years on the breeding and feeding of cattle.... Mr Mackenzie's main plea is for better bred, better handled, and more economically finished animals.... The chapters on dual purpose cattle, pedigree breeding, dairy shorthorns, and future possibilities are generally excellent.... Dr Marshall's chapter on physiology contains a great deal of valuable matter in small compass."—The Agricultural Correspondent of *The Glasgow Herald*

Manuring for Higher Crop Production. By E. J. RUSSELL, D.Sc., F.R.S., Director of the Rothamsted Experimental Station. Second edition, revised and extended. With 17 illustrations. Demy 8vo. 5s 6d net.

"An authentic and lucid record of modern researches into soils and manuring, with deductions and recommendations which the husbandman will find of great assistance.... The war period has given us no more opportune or valuable book for farmers."—*The Times*

British Grasses and their Employment in Agriculture. By S. F. ARMSTRONG, F.L.S. With 175 illustrations. Demy 8vo. 7s net.

"The Agricultural student, for whom primarily the volume has been written, will find in it a useful guide to his study of the grasses which form our meadows and pastures, and valuable help in their practical employment and treatment."
The Journal of Botany

The Naturalisation of Animals and Plants in New Zealand. By the Hon. GEO. M. THOMSON, M.L.C., F.N.Z.Inst. Royal 8vo. 42s net.

Typical Flies. A Photographic Atlas. By E. K. PEARCE. First Series. With 125 photographs. Royal 8vo. 10s net. Second Series. With 125 photographs. Royal 8vo. 15s net.

"The photographs are as good as any we have seen of this class of insect—a peculiarly difficult class to represent pictorially in any natural manner.... There are excellent short notes as to habitat, larval habits, and so on under the pictures.... We hope this volume may stimulate a wider interest in this fascinating group."
Nature on First Series

Organic Evolution. Outstanding difficulties and possible explanations. By LEONARD DARWIN, Hon. Sc.D. (Cantab.). Demy 8vo. 4s net.

Cambridge University Press
London, Fetter Lane, E.C. 4: C. F. Clay, Manager

THE ANNALS OF APPLIED BIOLOGY

The Annals of Applied Biology is conducted by the Association of Economic Biologists and is published by the Cambridge University Press. The Association exists to further the study of all aspects of Biology and to correlate pure science with practice. Meetings are held about eight times a year for informal discussion and the reading of papers.

The annual subscription to the Association, which includes a copy of the *Annals*, is 25*s*. (entrance fee 10*s*. 6*d*.), becomes due on Jan. 1st of each year and should be paid to the Treasurer.

The price of the *Annals* to non-members of the Association is £2. 0*s*. 0*d*. per volume; single parts 12*s*. Such subscriptions (payable in advance) and all communications respecting the publication should be sent to Mr C. F. Clay, Cambridge University Press, Fetter Lane, London, E.C. 4.

The publishers have appointed the University of Chicago Press Agents for the sale of *The Annals of Applied Biology* in the United States of America and Canada and have authorised them to charge the following prices: annual subscription $9.50, post free, single parts, $2.90.

Claims for missing numbers should be made within the month following that of regular publication.

Quotations can be given for binding cases and for binding subscribers' Sets; also for bound copies of back volumes.

Vols. I to VI of the *Annals* will be supplied at the following reduced rates:—To members of the Association, single volumes 16*s*., single parts 8*s*. 6*d*.; double numbers 14*s*. Complete sets I—VI £3. 12*s*. 0*d*. To non-members of the Association, single volumes £1. 0*s*. 0*d*., single parts 10*s*. 6*d*.; double numbers 17*s*. 6*d*. Complete sets I—VI £4. 10*s*. 0*d*. Part 4 vol. IV and Part 1 vol. VI can for the present be obtained for 5*s*.

Notice to Contributors.

MANUSCRIPT FOR PUBLICATION.

Contributors should address manuscripts on Botanical subjects to Mr W. B. Brierley, Rothamsted Experimental Station, Harpenden; and manuscripts on Zoological subjects to Mr D. Ward Cutler, Rothamsted Experimental Station, Harpenden. Papers submitted for publication should be typewritten and must conclude with a summary of contents. It is understood that they are not offered to any other Journal for prior or simultaneous publication.

Owing to the greatly increased cost of printing authors must condense their manuscript, confining themselves to the description of new and important results; and refrain from the inclusion of figures that are not absolutely essential. Tables should be reduced to the minimum and where possible be replaced by graphs.

It is suggested that the longer papers should not exceed 8000 words.

All but slight verbal alterations are assumed to have been made in the original manuscript and contributors will be responsible for any excess over the usual allowance.

Except under very special circumstances, determined by the Publications Committee, only text-figures will be paid for by the Association.

REFERENCES.

References to literature should be collected at the end of the paper and arranged alphabetically according to authors' names. Each should be accompanied by the date, title of Journal, volume, part and page; thus

10 SMITH, J. W. (1912). *Ann. App. Biol.* III. 2. 152–163.

In the text each reference should be indicated by its number enclosed in brackets.

ILLUSTRATIONS.

Illustrations and graphs accompanying the papers must be carefully drawn on smooth white Bristol board in Indian ink about twice the size of the finished block. Any lettering of these drawings should be lightly inserted in pencil. In all cases the magnification should be given.

SEPARATES.

Twenty-five separates without covers will be sent gratis to each contributor. Additional separates, with or without covers, may be purchased when ordered in advance.

PRINTED IN ENGLAND AT THE CAMBRIDGE UNIVERSITY PRESS

Vol. IX, No. 2 JUNE, 1922

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

PUBLICATIONS COMMITTEE

V. H. BLACKMAN
A. W. HILL
G. A. K. MARSHALL
A. D. IMMS
R. T. LEIPER

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, Manager
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 28, ESSEX STREET, LONDON, W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES AND CANADA)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

The Association of Economic Biologists

President

Prof. E. B. POULTON, M.A., D.Sc., LL.D., F.R.S.

Vice-Presidents

Prof. V. H. BLACKMAN, Sc.D., F.R.S.
G. A. K. MARSHALL, C.M.G., D.Sc.

Hon. Treasurer

A. D. IMMS, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Editors

W. B. BRIERLEY, D.Sc.
D. WARD CUTLER, M.A.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (General and Botanical)

W. B. BRIERLEY, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (Zoology)

J. WATERSTON, M.A., D.Sc.,
Nat. Hist. Museum,
South Kensington, S.W. 7

Council

W. LAWRENCE BALLS, Sc.D.
W. F. BEWLEY, D.Sc.
Prof. V. H. BLACKMAN, Sc.D., F.R.S.
F. T. BROOKS, M.A.
A. B. BRUCE, M.A.
E. J. BUTLER, D.Sc., C.I.E., M.B.
G. A. K. MARSHALL, C.M.G., D.Sc.
J. W. MUNRO, D.Sc.
S. A. NEAVE, D.Sc.
Prof. J. PERCIVAL, M.A.
Prof. J. H. PRIESTLEY, D.S.O., B.Sc.
E. J. RUSSELL, D.Sc., F.R.S.

CONTENTS OF Vol. IX, No. 2

PAGE

1. Bionomics of Weevils of the Genus *Sitona* injurious to Leguminous Crops in Britain. Part II. *Sitona hispidula* F., *S. sulcifrons* Thun and *S. crinita* Herbst. By Dorothy J. Jackson, F.E.S. (With 5 Text-figures and Plate III) 93
2. "Sleepy Disease" of the Tomato. By W. F. Bewley, D.Sc. (With Plates IV–VII) 116
3. Biological Studies of *Aphis rumicis* Linn. Reproduction on Varieties of *Vicia faba*. By J. Davidson, D.Sc. With a Statistical Appendix by R. A. Fisher, M.A. (With 1 Text-figure) 135
4. The Toxic Action of Traces of Coal Gas upon Plants. By J. H. Priestley 146
5. Common Scab of Potatoes. Part I. By W. A. Millard, B.Sc. (With Plates VIII, IX) 156
6. Additional Host Plants of *Oscinella frit*, Linn. among Grasses. By Norman Cunliffe, M.A. (Cantab.) 165
7. Studies in Bacteriosis. VI. *Bacillus carotovorus* as the cause of Soft-Rot in Cultivated Violets. By Margaret S. Lacey . . 169
8. Review 171
9. Report of the Council 173
10. Obituary. A. W. Bacot 175

E. & S. LIVINGSTONE, *Medical Publishers*
16 AND 17 TEVIOT PLACE: EDINBURGH

JUST PUBLISHED: Crown Quarto. 344 Pages. 105 Illustrations. 15s. net. Postage 1s.

PRACTICAL ZOOLOGY

FOR MEDICAL & JUNIOR STUDENTS

By J. D. F. GILCHRIST, M.A., D.Sc., Ph.D.
Professor of Zoology in the University of Cape Town
AND
C. VON BONDE, M.A.
Lecturer in Zoology in the University of Cape Town

The features of the book are the particularly clear arrangement of the text, with the right-hand pages left blank to enable the student to draw up his own diagrams, and the remarkably fine execution of the 105 illustrations, most of which are entirely original, and have been drawn from actual dissections.

CONTENTS

The Proteus Animalcule (*Amœba proteus*).—The Slipper Animalcule (*Paramecium*).—Monocystis.—Sponges (*Porifera*).—The Fresh-Water Polyp (*Hydra viridis*).—The Sea-Anemone (*Actinia*).—The Liver-Fluke (*Fasciola* or *Distoma hepatica*).—The Tape-Worm (*Taenia*).—The Earth-Worm (*Lumbricus terrestris*).—The Medicinal Leech (*Hirudo medicinalis*).—The Cockroach (*Periplaneta americana*).—The Fresh-Water Crayfish (*Astacus*) and The Cape Crawfish (*Jasus lalandii*).—The Snail (*Helix aspersa*).—The Fresh-Water Mussel (*Anodonta cygnea*) and the Common Marine Mussel (*Mytilus edulis*).—The Lancelet (*Amphioxus*, syn. *Branchiostoma lanceolatus*).—The Spiny Dogfish (*Acanthias*, syn. *Squalus*) or the Spotted Dogfish (*Scyllium*), and the Skate (*Raia*).—The Frog (*Rana*); the Platana or Clawed Toad (*Xenopus*).—The Pigeon (*Columba livia*, var. *domestica*).—The Rabbit (*Lepus cuniculus*).—A Classification of the above Types.—Index.

Cambridge University Press

Insect Pests and Fungus Diseases of Fruit and Hops. A Complete Manual for Growers. By P. J. Fryer, F.I.C., F.C.S. Crown 8vo. With 24 plates in natural colours and 305 original photographs and diagrams. 45s net.

"He has a particularly clear and orderly style of presenting his facts, giving all the information necessary to the practical man without forcing him to sift out unessential details.... Altogether the book deserves to become the standard reference work for growers."—*The Gardeners' Chronicle*

Cattle and the Future of Beef-Production in England. By K. J. J. Mackenzie, M.A. With a Preface and Chapter by F. H. A. Marshall, Sc.D. Demy 8vo. 7s 6d net.

"One of the best treatises issued in recent years on the breeding and feeding of cattle."—The Agricultural Correspondent of *The Glasgow Herald*

Manuring for Higher Crop Production. By E. J. Russell, D.Sc., F.R.S., Director of the Rothamsted Experimental Station. Second edition, revised and extended. With 17 illustrations. Demy 8vo. 5s 6d net.

"An authentic and lucid record of modern researches into soils and manuring, with deductions and recommendations which the husbandman will find of great assistance. . . . The war period has given us no more opportune or valuable book for farmers."—*The Times*

Cambridge University Press
London, Fetter Lane, E.C.4: C. F. Clay, Manager

THE ANNALS OF APPLIED BIOLOGY

The Annals of Applied Biology is conducted by the Association of Economic Biologists and is published by the Cambridge University Press. The Association exists to further the study of all aspects of Biology and to correlate pure science with practice. Meetings are held about eight times a year for informal discussion and the reading of papers.

The annual subscription to the Association, which includes a copy of the *Annals*, is 25*s*. (entrance fee 10*s*. 6*d*.), becomes due on Jan. 1st of each year and should be paid to the Treasurer.

The price of the *Annals* to non-members of the Association is £2. 0*s*. 0*d*. per volume; single parts 12*s*. Such subscriptions (payable in advance) and all communications respecting the publication should be sent to Mr C. F. Clay, Cambridge University Press, Fetter Lane, London, E.C. 4.

The publishers have appointed the University of Chicago Press Agents for the sale of *The Annals of Applied Biology* in the United States of America and Canada and have authorised them to charge the following prices: annual subscription $9.50, post free, single parts, $2.90.

Claims for missing numbers should be made within the month following that of regular publication.

Quotations can be given for binding cases and for binding subscribers' Sets; also for bound copies of back volumes.

Vols. I to VI of the *Annals* will be supplied at the following reduced rates:—To members of the Association, single volumes 16*s*., single parts 8*s*. 6*d*.; double numbers 14*s*. Complete sets I—VI £3. 12*s*. 0*d*. To non-members of the Association, single volumes £1. 0*s*. 0*d*., single parts 10*s*. 6*d*.; double numbers 17*s*. 6*d*. Complete sets I—VI £4. 10*s*. 0*d*. Part 4 vol. IV and Part 1 vol. VI can for the present be obtained for 5*s*.

Notice to Contributors.

MANUSCRIPT FOR PUBLICATION.

Contributors should address manuscripts on Botanical subjects to Mr W. B. Brierley, Rothamsted Experimental Station, Harpenden; and manuscripts on Zoological subjects to Mr D. Ward Cutler, Rothamsted Experimental Station, Harpenden. Papers submitted for publication should be typewritten and must conclude with a summary of contents. It is understood that they are not offered to any other Journal for prior or simultaneous publication.

Owing to the greatly increased cost of printing authors must condense their manuscript, confining themselves to the description of new and important results; and refrain from the inclusion of figures that are not absolutely essential. Tables should be reduced to the minimum and where possible be replaced by graphs.

It is suggested that the longer papers should not exceed 8000 words.

All but slight verbal alterations are assumed to have been made in the original manuscript and contributors will be responsible for any excess over the usual allowance.

Except under very special circumstances, determined by the Publications Committee, only text-figures will be paid for by the Association.

REFERENCES.

References to literature should be collected at the end of the paper and arranged alphabetically according to authors' names. Each should be accompanied by the date, title of Journal, volume, part and page; thus

10 SMITH, J. W. (1912). *Ann. App. Biol.* III. 2. 152–163.

In the text each reference should be indicated by its number enclosed in brackets.

ILLUSTRATIONS.

Illustrations and graphs accompanying the papers must be carefully drawn on smooth white Bristol board in Indian ink about twice the size of the finished block. Any lettering of these drawings should be lightly inserted in pencil. In all cases the magnification should be given.

SEPARATES.

Twenty-five separates without covers will be sent gratis to each contributor. Additional separates, with or without covers, may be purchased when ordered in advance.

PRINTED IN ENGLAND AT THE CAMBRIDGE UNIVERSITY PRESS

Vol. IX, Nos. 3 & 4 November, 1922

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

[illegible] 9 + 10 [illegible]

PUBLICATIONS COMMITTEE

V. H. BLACKMAN
A. W. HILL
G. A. K. MARSHALL
A. D. IMMS
R. T. LEIPER

1921-22

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, MANAGER
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES AND CANADA)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twenty-four Shillings net

The Association of Economic Biologists

President
PROF. E. B. POULTON, M.A., D.Sc., LL.D., F.R.S.

Vice-Presidents
PROF. V. H. BLACKMAN, Sc.D., F.R.S.
G. A. K. MARSHALL, C.M.G., D.Sc.

Hon. Treasurer
A. D. IMMS, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Editors
W. B. BRIERLEY, D.Sc.
D. WARD CUTLER, M.A.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (General and Botanical)
W. B. BRIERLEY, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (Zoology)
J. WATERSTON, M.A., D.Sc.,
Nat. Hist. Museum,
South Kensington, S.W. 7

Council
W. LAWRENCE BALLS, Sc.D.
W. F. BEWLEY, D.Sc.
PROF. V. H. BLACKMAN, Sc.D., F.R.S.
F. T. BROOKS, M.A.
A. B. BRUCE, M.A.
E. J. BUTLER, D.Sc., C.I.E., M.B.
G. A. K. MARSHALL, C.M.G., D.Sc.
J. W. MUNRO, D.Sc.
S. A. NEAVE, D.Sc.
PROF. J. PERCIVAL, M.A.
PROF. J. H. PRIESTLEY, D.S.O., B.Sc.
SIR JOHN RUSSELL, F.R.S.

CONTENTS OF VOL. IX, NOS. 3 & 4

PAGE

1. A Study of the Life-History of the Onion Fly (*Hylemyia antiqua*, Meigen). By KENNETH M. SMITH, A.R.C.S. (With Plates X and XI) 177
2. The Smut of Nachani or Ragi (*Eleusine coracana* Gaertn.). By G. S. KULKARNI. (With 2 Text-figures) 184
3. On the Young Larvae of *Lyctus brunneus* Steph. By A. M. ALTSON. F.E.S. (With 2 Text-figures) 187
4. Effect of High Root Temperature and Excessive Insolation upon Growth. By WINIFRED E. BRENCHLEY, D.Sc., assisted by KHARAK SINGH, M.A. (With 2 Text-figures) 197
5. Studies in Bacteriosis. VII. Comparison of the "Stripe Disease" with the "Grand Rapids Disease" of Tomato. By SYDNEY G. PAINE and MARGARET S. LACEY . 210
6. The Infestation of Fungus Cultures by Mites. (Its Nature and Control together with some Remarks on the Toxic Properties of Pyridine.) By SIBYL T. JEWSON, M.Sc. and F. TATTERSFIELD, B.Sc., F.I.C. (With 4 Text-figures) 213
7. On the Development of a Standardised Agar Medium for counting Soil Bacteria, with especial regard to the Repression of Spreading Colonies. By H. G. THORNTON. (With 13 Text-figures) 241
8. Studies on the Apple Canker Fungus. II. Canker Infection of Apple Trees through Scab Wounds. By S. P. WILTSHIRE, B.A., B.Sc. (With Plate XII) 275
9. The Insect and other Invertebrate Fauna of Arable Land at Rothamsted. By HUBERT M. MORRIS, M.Sc., F.E.S. (With 7 Text-figures) 282
10. On the Life-History of "Wireworms" of the Genus *Agriotes*, Esch., with some notes on that of *Athous haemorrhoidalis*, F. Part III. By A. W. RYMER ROBERTS, M.A. (With 1 Text-figure and Plates XIII, XIV) 306
11. The Accuracy of the Plating Method of estimating the Density of Bacterial Populations, with particular reference to the use of Thornton's Agar Medium with Soil Samples. By R. A. FISHER, M.A., H. G. THORNTON, B.A., and W. A. MACKENZIE, B.Sc. (With 2 Text-figures) 325

Cambridge University Press

Insect Pests and Fungus Diseases of Fruit and Hops. A Complete Manual for Growers. By P. J. FRYER, F.I.C., F.C.S. Crown 8vo. With 24 plates in natural colours and 305 original photographs and diagrams. 45s net.

"He has a particularly clear and orderly style of presenting his facts, giving all the information necessary to the practical man without forcing him to sift out unessential details.... Altogether the book deserves to become the standard reference work for growers."—*The Gardeners' Chronicle*

Cattle and the Future of Beef-Production in England. By K. J. J. MACKENZIE, M.A. With a Preface and Chapter by F. H. A. MARSHALL, Sc.D. Demy 8vo. 7s 6d net.

"One of the best treatises issued in recent years on the breeding and feeding of cattle."—The Agricultural Correspondent of *The Glasgow Herald*

Manuring for Higher Crop Production. By Sir JOHN RUSSELL, D.Sc., F.R.S., Director of the Rothamsted Experimental Station. Second edition, revised and extended. With 17 illustrations. Demy 8vo. 5s 6d net.

"An authentic and lucid record of modern researches into soils and manuring, with deductions and recommendations which the husbandman will find of great assistance. . . . The war period has given us no more opportune or valuable book for farmers."—*The Times*

British Grasses and their Employment in Agriculture. By S. F. ARMSTRONG, F.L.S. With 175 illustrations. Demy 8vo. 10s 6d net.

"The Agricultural student, for whom primarily the volume has been written, will find in it a useful guide to his study of the grasses which form our meadows and pastures, and valuable help in their practical employment and treatment."

The Journal of Botany

Zoology. An Elementary Text-Book. By Sir A. E. SHIPLEY, G.B.E., Sc.D., F.R.S., and E. W. MACBRIDE, D.Sc., F.R.S. **Fourth edition.** With 360 illustrations. Demy 8vo. 22s 6d net.

"The first edition of 'Shipley and MacBride' was very good, the second was better, and this, the third, is a marked improvement on the second....The authors have in unusual degree the gift of imparting information in a style at once compendious and lucid. The whole work affords an admirable introduction to a specialised course. It would also be useful to the amateur with a microscope....The diagrams are large and clear, and there is a good index."—*The Journal of Education*

The Naturalisation of Animals and Plants in New Zealand. By Hon. GEO. M. THOMSON, M.L.C., F.L.S., F.N.Z.Inst. Royal 8vo. 42s net.

"Mr Thomson has produced a remarkable study, more complete than any book on naturalisation with which we are acquainted."—*The Times Literary Supplement*

Growth and Form. By D'ARCY WENTWORTH THOMSON, C.B., D.Litt., F.R.S., Professor of Natural History in the University of St Andrews; Scientific Member of the Fishery Board for Scotland. Demy 8vo. With 408 illustrations. 30s net.

"A masterly book. It marks a big advance in science, and it will make other advances possible....Couched in a style that is always clear and dignified...a fresh revelation of the unity of Nature."—*Nature*

Cambridge University Press
London, Fetter Lane, E.C. 4: C. F. Clay, Manager

THE ANNALS OF APPLIED BIOLOGY

The Annals of Applied Biology is conducted by the Association of Economic Biologists and is published by the Cambridge University Press. The Association exists to further the study of all aspects of Biology and to correlate pure science with practice. Meetings are held about eight times a year for informal discussion and the reading of papers.

The annual subscription to the Association, which includes a copy of the *Annals*, is 25*s*. (entrance fee 10*s*. 6*d*.), becomes due on Jan. 1st of each year and should be paid to the Treasurer.

The price of the *Annals* to non-members of the Association is £2. 0*s*. 0*d*. per volume; single parts 12*s*. Such subscriptions (payable in advance) and all communications respecting the publication should be sent to Mr C. F. Clay, Cambridge University Press, Fetter Lane, London, E.C. 4.

The publishers have appointed the University of Chicago Press Agents for the sale of *The Annals of Applied Biology* in the United States of America and Canada and have authorised them to charge the following prices: annual subscription $9.50, post free; single parts, $2.90.

Claims for missing numbers should be made within the month following that of regular publication.

Quotations can be given for binding cases and for binding subscribers' Sets; also for bound copies of back volumes.

Vols. I to VI of the *Annals* will be supplied at the following reduced rates:—To members of the Association, single volumes 16*s*., single parts 8*s*. 6*d*.; double numbers 14*s*. Complete sets I—VI £3. 12*s*. 0*d*. To non-members of the Association, single volumes £1. 0*s*. 0*d*., single parts 10*s*. 6*d*.; double numbers 17*s*. 6*d*. Complete sets I—VI £4. 10*s*. 0*d*. Part 4 vol. IV and Part 1 vol. VI can for the present be obtained for 5*s*.

Notice to Contributors.

MANUSCRIPT FOR PUBLICATION.

Contributors should address manuscripts on Botanical subjects to Mr W. B. Brierley, Rothamsted Experimental Station, Harpenden; and manuscripts on Zoological subjects to Mr D. Ward Cutler, Rothamsted Experimental Station, Harpenden. Papers submitted for publication should be typewritten and must conclude with a summary of contents. It is understood that they are not offered to any other Journal for prior or simultaneous publication.

Owing to the greatly increased cost of printing authors must condense their manuscript, confining themselves to the description of new and important results; and refrain from the inclusion of figures that are not absolutely essential. Tables should be reduced to the minimum and where possible be replaced by graphs.

It is suggested that the longer papers should not exceed 8000 words.

All but slight verbal alterations are assumed to have been made in the original manuscript and contributors will be responsible for any excess over the usual allowance.

Except under very special circumstances, determined by the Publications Committee, only text-figures will be paid for by the Association.

REFERENCES.

References to literature should be collected at the end of the paper and arranged alphabetically according to authors' names. Each should be accompanied by the date, title of Journal, volume, part and page; thus

10 SMITH, J. W. (1912). *Ann. App. Biol.* III. 2. 152–163.

In the text each reference should be indicated by its number enclosed in brackets.

ILLUSTRATIONS.

Illustrations and graphs accompanying the papers must be carefully drawn on smooth white Bristol board in Indian ink about twice the size of the finished block. Any lettering of these drawings should be lightly inserted in pencil. In all cases the magnification should be given.

SEPARATES.

Twenty-five separates without covers will be sent gratis to each contributor. Additional separates, with or without covers, may be purchased when ordered in advance.

PRINTED IN ENGLAND AT THE CAMBRIDGE UNIVERSITY PRESS

MBL/WHOI LIBRARY
WH 17KU L